WEST Search History

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DATE: Thursday, October 14, 2004

Hide?	Set Name	Query	Hit Count
		DB=PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L9	thioredoxin reductase same (crystal or x-ray or atomic coordinates)	13
		DB=USPT,USOC,EPAB,JPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L8	thioredoxin reductase same (crystal or x-ray or atomic coordinates)	8
☐	L7	L6 and (crystal or x-ray or atomic coordinates)	73
☐	L6	thioredoxin reductase	207
		DB=PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L5	thioredoxin reductase same aureus	11
☐	L4	US-20030166843-A1.did.	1
☐	L3	US-20030166843-A1.did.	1
☐	L2	US-20030166843-A1.did.	1
		DB=USPT,USOC,EPAB,JPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L1	thioredoxin reductase same aureus	6

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Search Results - Record(s) 1 through 6 of 6 returned.

☐ 1. Document ID: US 6767536 B1

Using default format because multiple data bases are involved.

L1: Entry 1 of 6 File: USPT Jul 27, 2004

US-PAT-NO: 6767536
DOCUMENT-IDENTIFIER: US 6767536 B1

TITLE: Recombinant Staphylococcus thioredoxin reductase and inhibitors thereof useful as antimicrobial agents

DATE-ISSUED: July 27, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Aharonowitz; Yair	Hod Hasharon			IL
Borovok; Ilya	Ariel			IL
Cohen; Gerald	Raanana			IL
Uziel; Orit	Kfar-Saba			IL
Katz; Leonard	Oakland	CA		

US-CL-CURRENT: 424/93.42; 424/139.1, 424/165.1, 424/185.1, 424/237.1, 424/243.1, 424/94.1, 435/36, 435/7.33, 435/7.7, 435/91.1, 435/91.5, 435/91.51

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 2. Document ID: US 6559294 B1

L1: Entry 2 of 6 File: USPT May 6, 2003

US-PAT-NO: 6559294
DOCUMENT-IDENTIFIER: US 6559294 B1
**** See image for Certificate of Correction ****

TITLE: Chlamydia pneumoniae polynucleotides and uses thereof

DATE-ISSUED: May 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Griffais; Remy	Momtrouge			FR
Hoiseth; Susan K.	Fairport	NY		

Zagursky; Robert John	Victor	NY
Metcalf; Benjamin J.	Rochester	NY
Peek; Joel A.	Pittsford	NY
Sankaran; Banumathi	Penfield	NY
Fletcher; Leah Diane	Geneseo	NY

US-CL-CURRENT: 536/23.1; 435/320.1, 435/69.1, 435/70.1, 536/24.1

ABSTRACT:

The subject of the invention is the genomic sequence and the nucleotide sequences encoding polypeptides of Chlamydia pneumoniae, such as cellular envelope polypeptides, which are secreted or specific, or which are involved in metabolism, in the replication process or in virulence, polypeptides encoded by such sequences, as well as vectors including the said sequences and cells or animals transformed with these vectors. The invention also relates to transcriptional gene products of the Chlamydia pneumoniae genome, such as, for example, antisense and ribozyme molecules, which can be used to control growth of the microorganism. The invention also relates to methods of detecting these nucleic acids or polypeptides and kits for diagnosing Chlamydia pneumoniae infection. The invention also relates to a method of selecting compounds capable of modulating bacterial infection and a method for the biosynthesis or biodegradation of molecules of interest using the said nucleotide sequences or the said polypeptides. The invention finally comprises, pharmaceutical, in particular vaccine, compositions for the prevention and/or treatment of bacterial, in particular Chlamydia pneumoniae, infections.

13 Claims, 3 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 3

☐ 3. Document ID: US 6537773 B1

L1: Entry 3 of 6 File: USPT Mar 25, 2003

US-PAT-NO: 6537773
DOCUMENT-IDENTIFIER: US 6537773 B1

TITLE: Nucleotide sequence of the mycoplasma genitalium genome, fragments thereof, and uses thereof

DATE-ISSUED: March 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fraser; Claire M.	Potomac	MD		
Adams; Mark D.	N. Potomac	MD		
Gocayne; Jeannine D.	Silver Spring	MD		
Hutchison, III; Clyde A.	Chapel Hill	NC		
Smith; Hamilton O.	Towson	MD		
Venter; J. Craig	Potomac	MD		

White; Owen Gaithersburg MD

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 536/23.7, 536/24.32

ABSTRACT:

The present invention provides the nucleotide sequence of the entire genome of Mycoplasma genitalium, SEQ ID NO:1. The present invention further provides the sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use. In addition to the entire genomic sequence, the present invention identifies protein encoding fragments of the genome, and identifies, by position relative to two (2) genes known to flank the origin of replication, any regulatory elements which modulate the expression of the protein encoding fragments of the Mycoplasma genitalium genome.

44 Claims, 23 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 23

☐ 4. Document ID: US 6107068 A

L1: Entry 4 of 6 File: USPT Aug 22, 2000

US-PAT-NO: 6107068
DOCUMENT-IDENTIFIER: US 6107068 A

TITLE: Coenzyme A disulfide reductase, and inhibitors thereof useful as antimicrobial agents

DATE-ISSUED: August 22, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Katz; Leonard	Wheeling	IL		
Delcardayre; Stephen B.	Los Gatos	CA		
Davies; Julian E.	Vancouver			CA

US-CL-CURRENT: 435/189; 435/252.3, 435/320.1, 435/6, 536/23.2, 536/24.3

ABSTRACT:

An isolated and purified Coenzyme A disulfide reductase (CoADR) is provided. Oligonucleotides encoding the CoADR, vectors and host cells containing such oligonucleotides are also provided. In addition, antibodies reactive with the CoADR are provided, as are methods of isolating the CoADR, producing recombinant CoADR, using CoADR for screening compounds for CoADR-modulating activity, and detecting organisms which produce CoADR a test sample. Methods for identifying a gene encoding a CoADR are also provided.

24 Claims, 7 Drawing figures
Exemplary Claim Number: 1

Number of Drawing Sheets: 12

☐ 5. Document ID: US 20030166843 A1, WO 200177309 A2, AU 200149786 A

L1: Entry 5 of 6 File: DWPI Sep 4, 2003

DERWENT-ACC-NO: 2002-034237
DERWENT-WEEK: 200359

TITLE: Crystallizing Staphylococcus aureus thioredoxin reductase molecule or molecular complex by preparing purified thioredoxin reductase and crystallizing from solution comprising dimethyl sulfoxide and sodium formate

INVENTOR: BENSON, T E

PRIORITY-DATA: 2000US-195055P (April 6, 2000), 2001US-0825212 (April 3, 2001)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20030166843 A1	September 4, 2003		000	C07K001/00
WO 200177309 A2	October 18, 2001	E	147	C12N009/02
AU 200149786 A	October 23, 2001		000	C12N009/02

INT-CL (IPC): C07 K 1/00; C07 K 14/00; C07 K 17/00; C12 N 9/02; C12 Q 1/26; G06 F 17/50

ABSTRACTED-PUB-NO: WO 200177309A
BASIC-ABSTRACT:

NOVELTY - Crystallizing (M1) Staphylococcus aureus thioredoxin reductase molecule or molecular complex involves preparing purified S.aureus thioredoxin reductase at a concentration of about 1-50 mg/ml, and crystallizing the thioredoxin reductase from a solution at a pH of about 6-10 and comprising about 0-40 weight% dimethyl sulfoxide (DMSO) and about 100 mM-6 M sodium formate.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a molecule or molecular complex (I) comprising at least a portion of an S.aureus thioredoxin reductase or thioredoxin reductase-like FAD binding site, where the FAD binding site comprises the amino acids such as Ile 12 or Gly 13 defined in the specification, and is defined by a set of points having a root mean square deviation of less than about 1.1 Angstrom from points representing the backbone atoms of the amino acids as represented by the structure coordinates defined in the specification;

(2) a molecule or molecular complex (II) comprising at least a portion of an S.aureus thioredoxin reductase or thioredoxin reductase-like NADPH binding site, where the NADPH binding site comprises Cys 135, Cys 138, and the amino acids such as Asn 52 or Ala 136 defined in the specification, and is defined by a set of points having a root mean square deviation of less than about 0.8 Angstrom from points representing the backbone atoms of the amino acids as represented by the

structure coordinates defined in the specification;

(3) a molecule or molecular complex (III) that is structurally homologous to an S.aureus thioredoxin reductase molecule or molecular complex, where the molecule or molecular complex is represented by at least a portion of the structure coordinates defined in the specification;

(4) a scalable three dimensional configuration of points (IV), where at least a portion of the points or substantially all of the points are derived from structure coordinates of at least a portion of an S.aureus thioredoxin reductase molecule or molecular complex defined in the specification and comprises at least one of a thioredoxin reductase or thioredoxin reductase-like FAD binding site or an NADPH binding site;

(5) a machine-readable data storage medium (V) comprising a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using the data, is capable of displaying a graphical three-dimensional representation of at least one molecule or molecular complex selected from (I), (II) or (III);

(6) a machine-readable data storage medium (VI) comprising a data storage material encoded with a first set of machine readable data which, when combined with a second set of machine readable data, using a machine programmed with instructions for using the first set of data and the second set of data, can determine at least a portion of the structure coordinates corresponding to the second set of machine readable data, where the first set of data comprises a Fourier transform of at least a portion of the structural coordinates for S.aureus thioredoxin reductase defined in the specification, and the second set of data comprises an X-ray diffraction pattern of a molecule or molecular complex of unknown structure;

(7) obtaining (M2) structural information about a molecule or a molecular complex of unknown structure involves crystallizing the molecule or molecular complex, generating an X-ray diffraction pattern from the crystallized molecule or molecular complex, and applying at least a portion of the structure coordinates defined in the specification to the X-ray diffraction pattern to generate a three-dimensional electron density map of at least a portion of the molecule or molecular complex whose structure is unknown;

(8) homology modeling (M3) an S.aureus thioredoxin reductase homolog involves aligning the amino acid sequence S.aureus thioredoxin reductase homolog with an amino acid sequence S.aureus thioredoxin reductase and incorporating the sequence of the S.aureus thioredoxin reductase homolog into a model of S.aureus thioredoxin reductase derived from structure coordinates defined in the specification to yield a preliminary model of the S.aureus thioredoxin reductase homolog, subjecting the preliminary model to energy minimization to yield an energy minimized model, and remodeling regions of the energy minimized model where stereochemistry restraints are violated to yield a final model of the S.aureus thioredoxin reductase homolog;

(9) a computer-assisted method (M4) for identifying an inhibitor of S.aureus thioredoxin reductase activity involves supplying a computer modeling application with a set of structure coordinates of a molecule or molecular complex, where the molecule or molecular complex comprises at least a portion of an S.aureus thioredoxin reductase or thioredoxin reductase-like FAD or NADPH binding site, supplying the computer modeling application with a set of structure coordinates of a chemical entity, and determining whether the chemical entity is an inhibitor expected to bind to or interface with the molecule or molecular complex, where binding to or interfering with the molecule or molecular complex is indicative of potential inhibition of S.aureus thioredoxin reductase activity;

(10) making (M5) an inhibitor of S.aureus thioredoxin reductase activity, involves

chemically or enzymatically synthesizing a chemical entity to yield an inhibitor of S.aureus thioredoxin reductase activity, where the chemical entity has been identified during M4;

(11) an inhibitor (VII) of S.aureus thioredoxin reductase activity identified, designed or made by M4 or M5;

(12) a composition (VIII) comprising (VII) or its salt; and

(13) a crystal (IX) of S.aureus thioredoxin reductase.

ACTIVITY - None given.

MECHANISM OF ACTION - Inhibitor of S.aureus thioredoxin reductase activity (claimed). No supporting data is given.

USE - M1 is useful for crystallizing a S.aureus thioredoxin reductase molecule or molecular complex (claimed). The crystal obtained by M1 is useful for solving the structure of other molecules or molecular complexes, and designing inhibitors of S.aureus thioredoxin reductase. (VIII) is useful for preventing and treating S.aureus thioredoxin reductase mediated disease.

☐ 6. Document ID: US 20040161809 A1, WO 9945123 A1, US 6767536 B1

L1: Entry 6 of 6 File: DWPI Aug 19, 2004

DERWENT-ACC-NO: 1999-551044
DERWENT-WEEK: 200455

TITLE: A new thioredoxin reductase from Staphylococcus aureus

INVENTOR: AHARONOWITZ, Y; BOROVOK, I ; COHEN, G ; KATZ, L ; UZIEL, O

PRIORITY-DATA: 1998US-076525P (March 2, 1998), 1999US-0261301 (March 2, 1999), 2004US-0787887 (February 27, 2004)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20040161809 A1	August 19, 2004		000	G01N033/554
WO 9945123 A1	September 10, 1999	E	034	C12N015/53
US 6767536 B1	July 27, 2004		000	A01N063/00

INT-CL (IPC): A01 N 63/00; A61 K 38/54; A61 K 39/00; A61 K 39/09; A61 K 39/40; C07 K 16/40; C12 N 9/02; C12 N 15/53; C12 Q 1/18; C12 Q 1/26; C12 Q 1/68; G01 N 33/53; G01 N 33/554; G01 N 33/569

ABSTRACTED-PUB-NO: WO 9945123A
BASIC-ABSTRACT:

NOVELTY - An isolated Staphylococcus thioredoxin reductase (TrxB) polypeptide (P1) is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated polynucleotide encoding P1;

(2) a recombinant vector comprising N1 operably linked to control sequences that direct transcription of N1 for expression in a host cell;

(3) a host cell comprising the above vector;

(4) producing P1, comprising culturing the above host cell under expression conditions and recovering P1;

(5) an oligonucleotide probe capable of specifically hybridizing to a nucleic acid encoding P1, having about 8-50 contiguous nucleotides of N1;

(6) an antibody reactive with P1, particularly a monoclonal or polyclonal antibody;

(7) detecting Staphylococcus in a sample, comprising contacting the sample with the above probe and detecting hybridization complex;

(8) detecting Staphylococcus in a sample comprising contacting the sample with the above antibody and detecting antibody-P1 complex;

(9) detecting Staphylococcus in a sample comprising incubating the sample with a disulphide-containing substrate in the presence of thioredoxin and NADPH under substrate-reducing conditions to produce a detectable signal when P1 is present;

(10) a diagnostic kit comprising P1, the oligonucleotide probe of (5) or the antibody of (6);

(11) identifying a compound that modulates P1 activity, comprising:

(a) providing a P1 capable of catalyzing the specific reduction of thioredoxin with the concomitant oxidation of NADPH to NADP+;

(b) contacting a candidate compound with the P1 in the presence of thioredoxin, NADPH and a disulphide-containing substrate, preferably insulin or 5,5'-dithio-bis-2-nitrobenzoic acid; and

(c) monitoring the presence of free sulfhydryl groups as a measure of TrxB activity;

(12) a compound identified by the above method; and

(13) isolating P1 from a Staphylococcus cell culture extract, comprising:

(a) performing a protein precipitation step to yield a TrxB mixture;

(b) subjecting the mixture to gel-filtration chromatography;

(c) identifying fractions from (b) having TrxB activity; and

(d) performing anion exchange chromatography on the identified fractions to yield a product with greater TrxB activity than the mixture in (a).

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - thioredoxin reductase (TrxB) polypeptide inhibitors.

USE - P1 inhibitors are used as antimicrobials to treat a Staphylococcus, particularly S. aureus, infection (claimed).

ADVANTAGE - P1 inhibitors are antimicrobials to which, unlike most of those in the prior art, Staphylococcus has not developed a resistance.

Terms	Documents
thioredoxin reductase same aureus	6

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Search Results - Record(s) 1 through 8 of 8 returned.

☐ 1. Document ID: US 6784346 B1

Using default format because multiple data bases are involved.

L8: Entry 1 of 8 File: USPT Aug 31, 2004

US-PAT-NO: 6784346
DOCUMENT-IDENTIFIER: US 6784346 B1

TITLE: Value-added traits in grain and seed transformed with thioredoxin

DATE-ISSUED: August 31, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cho; Myeong-Je	Alameda	CA		
Lemaux; Peggy G.	Moraga	CA		
Buchanan; Bob B.	Berkeley	CA		
Wong; Joshua	San Francisco	CA		
Marx; Corina	Oakland	CA		

US-CL-CURRENT: 800/320; 800/278, 800/287, 800/295, 800/320.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 2. Document ID: US 6750046 B2

L8: Entry 2 of 8 File: USPT Jun 15, 2004

US-PAT-NO: 6750046
DOCUMENT-IDENTIFIER: US 6750046 B2

TITLE: Preparation of thioredoxin and thioredoxin reductase proteins on oil bodies

DATE-ISSUED: June 15, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moloney; Maurice M.	Calgary			CA
Dalmia; Bipin K.	San Diego	CA		

US-CL-CURRENT: 435/69.7; 435/189, 435/320.1, 435/419, 435/69.8, 536/23.4, 800/278, 800/281

ABSTRACT:

The present invention relates to the use of a class of genes called oil body protein genes that have unique features. The discovery of these features allowed the invention of methods for the production of recombinant proteins wherein a protein of interest can be easily separated from other host cell components. The invention is further exemplified by methods for exploitation of the unique characteristics of the oil body proteins and oil body genes for expression of polypeptides of interest in many organisms, particularly plant seeds. Said polypeptides include thioredoxin and/or thioredoxin reductase. The invention can also be modified to recover recombinant polypeptides fused to oil body proteins from non-plant host cells.

21 Claims, 46 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 38

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 3. Document ID: US 6689775 B2

L8: Entry 3 of 8 File: USPT Feb 10, 2004

US-PAT-NO: 6689775
DOCUMENT-IDENTIFIER: US 6689775 B2

TITLE: Uses of thioredoxin

DATE-ISSUED: February 10, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Powis; Garth	Tuscon	AZ		

US-CL-CURRENT: 514/208; 424/9.1, 424/9.361, 435/7.1, 514/183

ABSTRACT:

The present invention relates to the use of thioredoxin as, inter alia, a cell growth stimulator, as well as a screen for agents that are useful in reducing or preventing thioredoxin-associated apoptosis inhibition and agents that are useful in inhibiting thioredoxin stimulated cell growth.

3 Claims, 60 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 33

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 4. Document ID: US 6552060 B1

L8: Entry 4 of 8 File: USPT Apr 22, 2003

US-PAT-NO: 6552060
DOCUMENT-IDENTIFIER: US 6552060 B1

TITLE: Asymmetric disulfides and methods of using same

DATE-ISSUED: April 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kirkpatrick; D. Lynn	Emerald Park			CA

US-CL-CURRENT: 514/398; 514/396, 548/325.1

ABSTRACT:

The present invention is directed to a composition or formulation which includes an asymmetric disulfide which alone or in combination inhibits or interferes with cellular redox function, as well as a method of using same to restore normal cellular function. More specifically, the composition of the present invention interacts with, interferes with or inhibits abnormal cellular proliferation and restores or prevents inhibition of cellular apoptosis.

19 Claims, 16 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 11

☐ 5. Document ID: US 6380372 B1

L8: Entry 5 of 8 File: USPT Apr 30, 2002

US-PAT-NO: 6380372
DOCUMENT-IDENTIFIER: US 6380372 B1

TITLE: Barley gene for Thioredoxin and NADP-thioredoxin reductase

DATE-ISSUED: April 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cho; Myeong-Je	Alameda	CA		
del Val; Gregorio	El Cerrito	CA		
Caillau; Maxime	Verdun sur Garonne			FR
Lemaux; Peggy G.	Moraga	CA		
Buchanan; Bob B.	Berkeley	CA		

US-CL-CURRENT: 536/23.1; 435/183, 435/252.1, 435/320.1, 435/410, 435/69.1, 530/412, 800/278, 800/295, 800/298

ABSTRACT:

The present invention provides barley thioredoxin h nucleic acids and proteins. The barley thioredoxin h nucleic acid may be isolated or it may be an expression vector. The expression vector may be operably linked to a transcriptional regulatory sequence. The invention also provides for transgenic plants comprising recombinant barley thioredoxin h. The invention also provides methods of expressing and purifying barley thioredoxin h.

25 Claims, 21 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 19

☐ 6. Document ID: US 6372772 B1

L8: Entry 6 of 8 File: USPT Apr 16, 2002

US-PAT-NO: 6372772
DOCUMENT-IDENTIFIER: US 6372772 B1

TITLE: Inhibitors of redox signaling and methods of using same

DATE-ISSUED: April 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kirkpatrick; D. Lynn	Emerald Park			CA
Powis; Garth	Tucson	AZ		

US-CL-CURRENT: 514/396; 514/375, 514/397, 514/398, 514/399, 514/400

ABSTRACT:

The present invention is directed to a composition or formulation which inhibits or interferes with cellular redox function, and method of using same to restore normal cellular function. More specifically, the composition of the present invention interferes with or inhibits abnormal cellular proliferation and//restores or cellular apoptosis.

4 Claims, 6 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

☐ 7. Document ID: US 20030166843 A1, WO 200177309 A2, AU 200149786 A

L8: Entry 7 of 8 File: DWPI Sep 4, 2003

DERWENT-ACC-NO: 2002-034237
DERWENT-WEEK: 200359

TITLE: Crystallizing Staphylococcus aureus thioredoxin reductase molecule or molecular complex by preparing purified thioredoxin reductase and crystallizing from solution comprising dimethyl sulfoxide and sodium formate

INVENTOR: BENSON, T E

PRIORITY-DATA: 2000US-195055P (April 6, 2000), 2001US-0825212 (April 3, 2001)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20030166843 A1	September 4, 2003		000	C07K001/00
WO 200177309 A2	October 18, 2001	E	147	C12N009/02
AU 200149786 A	October 23, 2001		000	C12N009/02

INT-CL (IPC): C07 K 1/00; C07 K 14/00; C07 K 17/00; C12 N 9/02; C12 Q 1/26; G06 F 17/50

ABSTRACTED-PUB-NO: WO 200177309A
BASIC-ABSTRACT:

NOVELTY - Crystallizing (M1) Staphylococcus aureus thioredoxin reductase molecule or molecular complex involves preparing purified S.aureus thioredoxin reductase at a concentration of about 1-50 mg/ml, and crystallizing the thioredoxin reductase from a solution at a pH of about 6-10 and comprising about 0-40 weight% dimethyl sulfoxide (DMSO) and about 100 mM-6 M sodium formate.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a molecule or molecular complex (I) comprising at least a portion of an S.aureus thioredoxin reductase or thioredoxin reductase-like FAD binding site, where the FAD binding site comprises the amino acids such as Ile 12 or Gly 13 defined in the specification, and is defined by a set of points having a root mean square deviation of less than about 1.1 Angstrom from points representing the backbone atoms of the amino acids as represented by the structure coordinates defined in the specification;

(2) a molecule or molecular complex (II) comprising at least a portion of an S.aureus thioredoxin reductase or thioredoxin reductase-like NADPH binding site, where the NADPH binding site comprises Cys 135, Cys 138, and the amino acids such as Asn 52 or Ala 136 defined in the specification, and is defined by a set of points having a root mean square deviation of less than about 0.8 Angstrom from points representing the backbone atoms of the amino acids as represented by the structure coordinates defined in the specification;

(3) a molecule or molecular complex (III) that is structurally homologous to an S.aureus thioredoxin reductase molecule or molecular complex, where the molecule or molecular complex is represented by at least a portion of the structure coordinates defined in the specification;

(4) a scalable three dimensional configuration of points (IV), where at least a portion of the points or substantially all of the points are derived from structure coordinates of at least a portion of an S.aureus thioredoxin reductase molecule or molecular complex defined in the specification and comprises at least one of a

thioredoxin reductase or thioredoxin reductase-like FAD binding site or an NADPH binding site;

(5) a machine-readable data storage medium (V) comprising a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using the data, is capable of displaying a graphical three-dimensional representation of at least one molecule or molecular complex selected from (I), (II) or (III);

(6) a machine-readable data storage medium (VI) comprising a data storage material encoded with a first set of machine readable data which, when combined with a second set of machine readable data, using a machine programmed with instructions for using the first set of data and the second set of data, can determine at least a portion of the structure coordinates corresponding to the second set of machine readable data, where the first set of data comprises a Fourier transform of at least a portion of the structural coordinates for S.aureus thioredoxin reductase defined in the specification, and the second set of data comprises an X-ray diffraction pattern of a molecule or molecular complex of unknown structure;

(7) obtaining (M2) structural information about a molecule or a molecular complex of unknown structure involves crystallizing the molecule or molecular complex, generating an X-ray diffraction pattern from the crystallized molecule or molecular complex, and applying at least a portion of the structure coordinates defined in the specification to the X-ray diffraction pattern to generate a three-dimensional electron density map of at least a portion of the molecule or molecular complex whose structure is unknown;

(8) homology modeling (M3) an S.aureus thioredoxin reductase homolog involves aligning the amino acid sequence S.aureus thioredoxin reductase homolog with an amino acid sequence S.aureus thioredoxin reductase and incorporating the sequence of the S.aureus thioredoxin reductase homolog into a model of S.aureus thioredoxin reductase derived from structure coordinates defined in the specification to yield a preliminary model of the S.aureus thioredoxin reductase homolog, subjecting the preliminary model to energy minimization to yield an energy minimized model, and remodeling regions of the energy minimized model where stereochemistry restraints are violated to yield a final model of the S.aureus thioredoxin reductase homolog;

(9) a computer-assisted method (M4) for identifying an inhibitor of S.aureus thioredoxin reductase activity involves supplying a computer modeling application with a set of structure coordinates of a molecule or molecular complex, where the molecule or molecular complex comprises at least a portion of an S.aureus thioredoxin reductase or thioredoxin reductase-like FAD or NADPH binding site, supplying the computer modeling application with a set of structure coordinates of a chemical entity, and determining whether the chemical entity is an inhibitor expected to bind to or interface with the molecule or molecular complex, where binding to or interfering with the molecule or molecular complex is indicative of potential inhibition of S.aureus thioredoxin reductase activity;

(10) making (M5) an inhibitor of S.aureus thioredoxin reductase activity, involves chemically or enzymatically synthesizing a chemical entity to yield an inhibitor of S.aureus thioredoxin reductase activity, where the chemical entity has been identified during M4;

(11) an inhibitor (VII) of S.aureus thioredoxin reductase activity identified, designed or made by M4 or M5;

(12) a composition (VIII) comprising (VII) or its salt; and

(13) a crystal (IX) of S.aureus thioredoxin reductase.

ACTIVITY - None given.

MECHANISM OF ACTION - Inhibitor of S.aureus thioredoxin reductase activity (claimed). No supporting data is given.

USE - M1 is useful for crystallizing a S.aureus thioredoxin reductase molecule or molecular complex (claimed). The crystal obtained by M1 is useful for solving the structure of other molecules or molecular complexes, and designing inhibitors of S.aureus thioredoxin reductase. (VIII) is useful for preventing and treating S.aureus thioredoxin reductase mediated disease.

☐ 8. Document ID: WO 200112657 A2, AU 200069872 A

L8: Entry 8 of 8 File: DWPI Feb 22, 2001

DERWENT-ACC-NO: 2001-202856
DERWENT-WEEK: 200120

TITLE: Expression of heterologous selenoproteins in bacterial cells, involves insertion of selenocysteine by manipulating bacterial insertion machinery and engineered selenocysteine insertion sequence elements

INVENTOR: ARNER, E S J; BOCK, A ; HOLMGREN, A

PRIORITY-DATA: 1999US-149033P (August 16, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 200112657 A2	February 22, 2001	E	051	C07K014/00
AU 200069872 A	March 13, 2001		000	C07K014/00

INT-CL (IPC): C07 K 14/00

ABSTRACTED-PUB-NO: WO 200112657A
BASIC-ABSTRACT:

NOVELTY - Producing (M1) a mammalian or other heterologous selenoprotein (SP) in bacterial cells, comprising transforming bacterial cells with nucleic acid (I) having coding sequence for SP, bacterial selenocysteine insertion sequence (SECIS) and regulatory sequences for expression of SP in bacterial cells, and culturing the cells for overexpression of selA, selB and selC so that SP is produced, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) SP produced by (M1);

(2) a bacterial host cell (II) which comprises a coding sequence for SP, SECIS, and regulatory sequences for expression of the encoded SP in bacterial host cells, and which contains nucleic acid for overexpression of selA, selB and selC; and

(3) a nucleic acid construct (III) for producing a mammalian or other heterologous SP in a bacterial host cell, comprising a coding sequence for SP, SECIS, and

regulatory sequences for expression of encoded SP in bacterial host cells.

USE - For producing SP, thioredoxin reductase in bacterial host cells such as Escherichia coli (claimed). Introduction of a selenocysteine and/or replacing a cysteine with selenocysteine provides for enhanced resolution in X-ray crystallography, for PET studies, for probing redox activities and for introduction of highly energetic selenium isotopes e.g. 75Se to enable radiochemical methods for the protein of interest.

ADVANTAGE - Mammalian proteins e.g. thioredoxin reductase can be produced as a recombinant selenoprotein in Escherichia coli without any difficulties associated with current techniques for purification of small amounts of the protein from animal tissue. Using the novel method it is now possible to produce mutant enzymes still carrying the selenocysteine residue but not being mutated in other key residues.

Terms	Documents
thioredoxin reductase same (crystal or x-ray or atomic coordinates)	8

Hit List

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Search Results - Record(s) 1 through 13 of 13 returned.

☐ 1. Document ID: US 20040180038 A1

Using default format because multiple data bases are involved.

L9: Entry 1 of 13 File: PGPB Sep 16, 2004

PGPUB-DOCUMENT-NUMBER: 20040180038
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040180038 A1

TITLE: Effectors of innate immunity determination

PUBLICATION-DATE: September 16, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hancock, Robert E. W.	Vancouver		CA	
Finlay, B. Brett	Richmond		CA	
Scott, Monisha Gough	Vancouver		CA	
Bowdish, Dawn	Vancouver		CA	
Rosenberger, Carrie Melissa	Vancouver		CA	
Powers, Jon-Paul Steven	Vancouver		CA	

US-CL-CURRENT: 424/93.2; 435/6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 2. Document ID: US 20040109875 A1

L9: Entry 2 of 13 File: PGPB Jun 10, 2004

PGPUB-DOCUMENT-NUMBER: 20040109875
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040109875 A1

TITLE: Pro-apoptotic bacterial vaccines to enhance cellular immune responses

PUBLICATION-DATE: June 10, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kernodle, Douglas S.	Brentwood	TN	US	
Bochan, Markian R	Nashville	TN	US	

US-CL-CURRENT: 424/200.1; 435/252.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 3. Document ID: US 20040014040 A1

L9: Entry 3 of 13 File: PGPB Jan 22, 2004

PGPUB-DOCUMENT-NUMBER: 20040014040
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040014040 A1

TITLE: Cardiotoxin molecular toxicology modeling

PUBLICATION-DATE: January 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mendrick, Donna	Gaithersburg	MD	US	
Porter, Mark	Gaithersburg	MD	US	
Johnson, Kory	Gaithersburg	MD	US	
Higgs, Brandon	Gaithersburg	MD	US	
Castle, Arthur	Gaithersburg	MD	US	
Elashoff, Michael	Gaithersburg	MD	US	

US-CL-CURRENT: 435/6; 702/20

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 4. Document ID: US 20040001803 A1

L9: Entry 4 of 13 File: PGPB Jan 1, 2004

PGPUB-DOCUMENT-NUMBER: 20040001803
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040001803 A1

TITLE: Effectors of innate immunity determination

PUBLICATION-DATE: January 1, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hancock, Robert E.W.	Vancouver		CA	
Finlay, B. Brett	Richmond		CA	
Gough Scott, Monisha	Vancouver		CA	
Bowdish, Dawn	Vancouver		CA	
Rosenberger, Carrie Melissa	Vancouver		CA	
Steven Powers, Jon-Paul	Vancouver		CA	

US-CL-CURRENT: 424/85.2; 435/6, 514/14, 514/15, 514/16, 530/326, 530/327, 530/328

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 5. Document ID: US 20030223980 A1

L9: Entry 5 of 13 File: PGPB Dec 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030223980
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030223980 A1

TITLE: Uses of thioredoxin

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Powis, Garth	Tucson	AZ	US	

US-CL-CURRENT: 424/94.4; 435/7.23

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 6. Document ID: US 20030176512 A1

L9: Entry 6 of 13 File: PGPB Sep 18, 2003

PGPUB-DOCUMENT-NUMBER: 20030176512
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030176512 A1

TITLE: Asymmetric disulfides and methods of using same

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kirkpatrick, D. Lynn	Tuscon	AZ	US	

US-CL-CURRENT: 514/707

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 7. Document ID: US 20030167524 A1

L9: Entry 7 of 13 File: PGPB Sep 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030167524
PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030167524 A1

TITLE: Methods for the production of multimeric protein complexes, and related compositions

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Rooijen, Gijs Van	Alberta	CA	CA	
Zaplachinski, Steven	Alberta	CA	CA	
Heifetz, Peter-Bernard	San Diego	CA	US	
Briggs, Steven	Del Mar	CA	US	
Dalmia, Bipin Kumar	San Diego		US	
Val, Greg Del	San Diego		US	

US-CL-CURRENT: 800/281; 435/419

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 8. Document ID: US 20030166843 A1

L9: Entry 8 of 13 File: PGPB Sep 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030166843
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030166843 A1

TITLE: Crystallization and structure determination of staphylococcus aureus thioredoxin reductase

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Benson, Timothy E.	Kalamazoo	MI	US	

US-CL-CURRENT: 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 9. Document ID: US 20030150010 A1

L9: Entry 9 of 13 File: PGPB Aug 7, 2003

PGPUB-DOCUMENT-NUMBER: 20030150010
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030150010 A1

TITLE: Barley gene for thioredoxin and NADP-thioredoxin reductase

PUBLICATION-DATE: August 7, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cho, Myeong-Je	Alameda	CA	US	
del Val, Gregorio	El Cerrito	CA	US	
Caillau, Maxime	Verdun sur Garonne	CA	FR	
Lemaux, Peggy G.	Moraga	CA	US	
Buchanan, Bob B.	Berkeley		US	

US-CL-CURRENT: 800/278

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 10. Document ID: US 20030093832 A1

L9: Entry 10 of 13 File: PGPB May 15, 2003

PGPUB-DOCUMENT-NUMBER: 20030093832
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030093832 A1

TITLE: Methods for the production of multimeric immunoglobulins, and related compositions

PUBLICATION-DATE: May 15, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Szarka, Steven	Calgary		CA	
Van Rooijen, Gijs	Calgary		CA	
Moloney, Maurice	Calgary		CA	

US-CL-CURRENT: 800/281; 435/419, 530/388.26, 800/288

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 11. Document ID: US 20020088025 A1

L9: Entry 11 of 13 File: PGPB Jul 4, 2002

PGPUB-DOCUMENT-NUMBER: 20020088025
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020088025 A1

TITLE: Preparation of thioredoxin and thioredoxin reductase proteins on oil bodies

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Moloney, Maurice M.	Calgary	CA	CA	
Dalmia, Bipin K.	San Diego		US	

US-CL-CURRENT: 800/288; 435/69.8, 536/23.4, 800/291

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 12. Document ID: US 20020055131 A1

L9: Entry 12 of 13 File: PGPB May 9, 2002

PGPUB-DOCUMENT-NUMBER: 20020055131
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020055131 A1

TITLE: Uses of thioredoxin

PUBLICATION-DATE: May 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Powis, Garth	Tucson	AZ	US	

US-CL-CURRENT: 435/7.23; 435/40.5, 514/1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 13. Document ID: US 20020037303 A1

L9: Entry 13 of 13 File: PGPB Mar 28, 2002

PGPUB-DOCUMENT-NUMBER: 20020037303
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020037303 A1

TITLE: Thioredoxin and thioredoxin reductase containing oil body based products

PUBLICATION-DATE: March 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Deckers, Harm M.	Calgary	CA	CA	
Rooijen, Gijs van	Calgary		CA	
Boothe, Joseph	Calgary		CA	
Goll, Janis	Calgary		CA	
Moloney, Maurice M.	Calgary		CA	
Dalmia, Bipin K.	San Diego		US	

US-CL-CURRENT: 424/401

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KMC | Draw D

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Terms	Documents
thioredoxin reductase same (crystal or x-ray or atomic coordinates)	13

Display Format: - Change Format

Previous Page Next Page Go to Doc#

```
=> file .nash
=> s thioredoxin reductase and aureus
L1             7 FILE MEDLINE
L2            16 FILE CAPLUS
L3            11 FILE SCISEARCH
L4             5 FILE LIFESCI
L5             7 FILE BIOSIS
L6             6 FILE EMBASE

TOTAL FOR ALL FILES
L7            52 THIOREDOXIN REDUCTASE AND AUREUS

=> s l7 not 2001-2004/py
TOTAL FOR ALL FILES
L14           27 L7 NOT 2001-2004/PY

=> dup rem l14
PROCESSING COMPLETED FOR L14
L15            8 DUP REM L14 (19 DUPLICATES REMOVED)

=> d ibib abs 1-8

L15  ANSWER 1 OF 8  SCISEARCH  COPYRIGHT (c) 2004 The Thomson Corporation.  on
     STN
ACCESSION NUMBER:    2000:608106  SCISEARCH
THE GENUINE ARTICLE: 341MC
TITLE:               Formation of the cystine between cysteine 225 and cysteine
                     462 from ribonucleoside diphosphate reductase is
                     kinetically competent
AUTHOR:              Erickson H K (Reprint)
CORPORATE SOURCE:    UNIV UTAH, DEPT CHEM, 315 S 1400 E, ROOM 2020, SALT LAKE
                     CITY, UT 84112 (Reprint); UNIV CALIF SAN DIEGO, DEPT CHEM,
                     LA JOLLA, CA 92093
COUNTRY OF AUTHOR:   USA
SOURCE:              BIOCHEMISTRY, (8 AUG 2000) Vol. 39, No. 31, pp. 9241-9250.
                     Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW,
                     WASHINGTON, DC 20036.
                     ISSN: 0006-2960.
DOCUMENT TYPE:       Article; Journal
FILE SEGMENT:        LIFE
LANGUAGE:            English
REFERENCE COUNT:     36
                    *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB       Participation of the formation of the cystine between cysteine 225 and
     cysteine 462 in the R1 protein to the enzymatic mechanism of aerobic
     ribonucleoside diphosphate reductase from Escherichia coli has been
     examined by use of rapid quenching and site-directed immunochemistry.
     Prereduced ribonucleotide reductase in the presence of ATP was mixed with
     CDP in a quench flow apparatus. The reaction was terminated with a
     solution of acetic acid and N-ethylmaleimide. The protein was precipitated
     and digested with chymotrypsin and the proteinase from Staphylococcus
     aureus strain V8 in the presence of N-ethylmaleimide to yield the
     peptide SS[S-(N-ethylsuccinimid-2-yl)cysteinyl]VLIE containing cysteine
     225 and the mixed disulfide between the peptide SSCVLIE and the peptide
     IALCTL containing cysteine 462. These two peptides were retrieved together
     from the digest by immunoadsorption. The affinity-purified peptides were
     modified at their amino termini with the fluorescent reagent
     6-aminoquinolyl-N-hydroxysuccimidyl carbamate and submitted to
     high-pressure liquid chromatography. The areas of the respective peaks of
     fluorescence corresponding to the S-(N-ethylsuccimidyl) peptide, and the
     mixed disulfide were used to determine the percentage of the cystine that
     had formed during each interval. The rate constant for the formation of
     the cystine following the association of free, fully reduced
     ribonucleotide reductase with the reactant CDP was 8 s(-1). Because only
     50% of the active sites participated in this pre-steady state reaction,
     the maximum steady-state rate consistent with the involvement of this
     cystine in the enzymatic reaction would be 4 s(-1). Since the turnover
     number of the enzyme under the same conditions in a steady state assay was
     only 1 s(-1), the formation of the cystine between these two cysteines is
     kinetically competent.
```

L15 ANSWER 2 OF 8 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2000074536 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10606519
TITLE: The essential catalytic redox couple in arsenate reductase from Staphylococcus **aureus**.
AUTHOR: Messens J; Hayburn G; Desmyter A; Laus G; Wyns L
CORPORATE SOURCE: Dienst Ultrastructuur, Vlaams Interuniversitair instituut voor Biotechnologie, Vrije Universiteit Brussel, Belgium.. jmessens@vub.ac.be
SOURCE: Biochemistry, (1999 Dec 21) 38 (51) 16857-65.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000131
Last Updated on STN: 20020420
Entered Medline: 20000119

AB Arsenate reductase (ArsC) encoded by Staphylococcus **aureus** arsenic-resistance plasmid pI258 reduces intracellular As(V) (arsenate) to the more toxic As(III) (arsenite), which is subsequently extruded from the cell. ArsC couples to thioredoxin, **thioredoxin reductase**, and NADPH to be enzymatically active. A novel purification method leads to high production levels of highly pure enzyme. A reverse phase method was introduced to systematically analyze and control the oxidation status of the enzyme. The essential cysteinyl residues and redox couple in arsenate reductase were identified by a combination of site-specific mutagenesis and endoprotease-digest mass spectroscopy analysis. The secondary structures, as determined with CD, of wild-type ArsC and its Cys mutants showed a relatively high helical content, independent of the redox status. Mutation of Cys 10, 82, and 89 led to redox-inactive enzymes. ArsC was oxidized in a single catalytic cycle and subsequently digested with endoproteinases ArgC, AspN, and GluC. From the peptide-mass profiles, cysteines 82 and 89 were identified as the redox couple of ArsC necessary to reduce arsenate to arsenite.

L15 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:779011 CAPLUS
DOCUMENT NUMBER: 132:274994
TITLE: Antibiotic resistance as a stress response: complete sequencing of a large number of chromosomal loci in Staphylococcus **aureus** strain COL that impact on the expression of resistance to methicillin
AUTHOR(S): de Lencastre, H.; Wu, S. W.; Pinho, M. G.; Ludovice, A. M.; Filipe, S.; Gardete, S.; Sobral, R.; Gill, S.; Chung, M.; Tomasz, A.
CORPORATE SOURCE: Lab Microbiol., Rockefeller Univ., New York, NY, 10021, USA
SOURCE: Microbial Drug Resistance (Larchmont, New York) (1999), 5(3), 163-175
CODEN: MDREFJ; ISSN: 1076-6294
PUBLISHER: Mary Ann Liebert, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Tn551 inactivation has identified several determinants fem or auxiliary genes that, in addn. to the mecA gene, are also crit. for the expression of high-level and homogeneous resistance to methicillin. Genetic and/or biochem. anal. has shown that of the nearly dozen aux mutations described so far, most are in genes involved in cell wall synthesis (murE, pbp2, glmM, glnR, femA/B, llm, etc.) or incomplex regulatory functions (sigmaB), suggesting that optimal expression of resistance may involve the cooperative functioning of a no. of genes in cell wall metab. as well as stress response. The exact mechanism of these functions is not known. In an attempt to explore this unusual aspect of methicillin resistance more fully, a Tn551 transposon library, constructed in the background of the highly and homogeneously methicillin-resistant Staphylococcus **aureus** strain COL, was screened for all independent insertional mutants in which the level of methicillin resistance of the parental strain (MIC, 1,600 .mu.g/mL) was reduced by at least 15-fold and up to

500-fold. The sequencing of 21 Tn551-inactivated genes and their vicinities in 23 new auxiliary mutants that were studied before are now described. Using the inverted polymerase chain reaction (IPCR), the authors amplified fragments corresponding to the right and left junction of the Tn551 insertions, which were then sequenced by primer walking. The two largest groups of these new auxiliary genes encoded either proteins of unknown functions(6 genes) or showed homol. with genes encoding proteins involved with putative sensory/regulatory activities (7 genes:protein kinases, ABC transporters, and a catabolite control protein). Sequencing upstream and downstream allowed the identification of a no. of addnl. open reading frames, some of which may also include functions relevant for the expression of antibiotic resistance.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 4 OF 8 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 97342719 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9199416
TITLE: Increased intracellular survival of Mycobacterium smegmatis containing the Mycobacterium leprae thioredoxin-**thioredoxin reductase** gene.
AUTHOR: Wieles B; Ottenhoff T H; Steenwijk T M; Franken K L; de Vries R R; Langermans J A
CORPORATE SOURCE: Department of Immunohematology and Blood Bank, Leiden University Hospital, The Netherlands.. Wieles@imm.unibe.ch
SOURCE: Infection and immunity, (1997 Jul) 65 (7) 2537-41.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970805
Last Updated on STN: 19970805
Entered Medline: 19970721

AB The thioredoxin (Trx) system of Mycobacterium leprae is expressed as a single hybrid protein containing **thioredoxin reductase** (TR) at its N terminus and Trx at its C terminus. This hybrid Trx system is unique to M. leprae, since in all other organisms studied to date, including other mycobacteria, both TR and Trx are expressed as two separate proteins. Because Trx has been shown to scavenge reactive oxygen species, we have investigated whether the TR-Trx gene product can inhibit oxygen-dependent killing of mycobacteria by human mononuclear phagocytes and as such could contribute to mycobacterial virulence. The gene encoding M. leprae TR-Trx was cloned into the apathogenic, fast-growing bacterium Mycobacterium smegmatis. Recombinant M. smegmatis containing the gene encoding TR-Trx was killed to a significantly lesser extent than M. smegmatis containing the identical vector with either no insert or a control M. leprae construct unrelated to TR-Trx. Upon phagocytosis, M. smegmatis was shown to be killed predominantly by oxygen-dependent macrophage-killing mechanisms. Coinfection of M. smegmatis expressing the gene encoding TR-Trx together with Staphylococcus **aureus**, which is known to be killed via oxygen-dependent microbicidal mechanisms, revealed that the TR-Trx gene product interferes with the intracellular killing of this bacterium. A similar coinfection with Streptococcus pyogenes, known to be killed by oxygen-independent mechanisms, showed that the TR-Trx gene product did not influence the oxygen-independent killing pathway. The data obtained in this study suggest that the Trx system of M. leprae can inhibit oxygen-dependent killing of intracellular bacteria and thus may represent one of the mechanisms by which M. leprae can deal with oxidative stress within human mononuclear phagocytes.

L15 ANSWER 5 OF 8 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 94271784 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8003493
TITLE: Arsenate reductase of Staphylococcus **aureus** plasmid pI258.
AUTHOR: Ji G; Garber E A; Armes L G; Chen C M; Fuchs J A; Silver S
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago 60612-7344.
SOURCE: Biochemistry, (1994 Jun 14) 33 (23) 7294-9.

Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940729
Last Updated on STN: 20020420
Entered Medline: 19940721

AB Arsenate reductase encoded by Staphylococcus **aureus** arsenic-resistance plasmid pI258 was overproduced in Escherichia coli and purified. The purified enzyme reduced radioactive arsenate to arsenite when coupled to thioredoxin, **thioredoxin reductase**, and NADPH. NADPH oxidation coupled to arsenate reduction also required thioredoxin and **thioredoxin reductase**. Glutaredoxin and reduced glutathione did not stimulate arsenate reduction. NADPH oxidation showed Michaelis-Menten kinetics with a Km of 1 microM AsO4(3-) and an apparent Vmax of 200 nmol/min per mg of protein. At high substrate concentration (above 1 mM AsO4(3-), a secondary rise in the reaction rate was observed, with a Km of 2 mM and an apparent Vmax of 450 nmol/min per mg of protein. This secondary rise also occurred upon addition of phosphate or nitrate (which were not substrates for the enzyme). Arsenite (the product of the enzyme), tellurite, and antimonite [Sb(III)] were inhibitors. Selenate (but not selenite or sulfate) was a substrate for reductase-dependent NADPH oxidation, with an apparent Km of 13 mM SeO4(2-). Arsenate reductase was purified as a monomer of 14.5 kDa, consistent with the DNA sequence. Electrospray mass spectrometry showed two molecular masses of 14,810.5 and 14,436.0 Da, suggesting that 70% of the purified protein lacked the N-terminal three amino acids; HPLC coupled to electrospray mass spectroscopy of protease digest products confirmed this conclusion and verified the entire amino acid sequence.

L15 ANSWER 6 OF 8 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 91249849 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2040309
TITLE: Characterization and primary structure of a second thioredoxin from the green alga, Chlamydomonas reinhardtii.
AUTHOR: Decottignies P; Schmitter J M; Dutka S; Jacquot J P; Miginiac-Maslow M
CORPORATE SOURCE: Laboratoire de Physiologie Vegetale Moleculaire, Universite Paris-Sud, Orsay, France.
SOURCE: European journal of biochemistry / FEBS, (1991 Jun 1) 198 (2) 505-12.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199107
ENTRY DATE: Entered STN: 19910728
Last Updated on STN: 19910728
Entered Medline: 19910710

AB A second thioredoxin, Ch1, distinct from the one recently reported [Decottignies, P., Schmitter, J.M., Jacquot, J. P., Dutka, S., Picaud, A. & Gadal, P. (1990) Arch, Biochem. Biophys. 280, 112-121] has been purified from the green alga, Chlamydomonas reinhardtii, and its functional and structural properties investigated. Its activity in various enzymatic assays has been compared with the activities of different plant thioredoxins (Ch2 from C. reinhardtii and spinach m and f). Ch1 cannot serve as a substrate for Escherichia coli **thioredoxin reductase**, but can be reduced by spinach ferredoxin-**thioredoxin reductase**. It is less efficient than its spinach counterpart in the activation of corn leaf NADP-dependent malate dehydrogenase by light or dithiothreitol, and it only activates spinach fructose-1,6-bisphosphatase at very high concentrations. The complete primary structure of C. reinhardtii thioredoxin Ch1 was determined by automated Edman degradation of the intact protein and of peptides derived from trypsin, chymotrypsin and Staphylococcus **aureus** V8 protease digestions. When needed, peptide masses were verified by plasma desorption mass spectrometry. Ch1 consists of a polypeptide of 111 amino acids (11634 Da) and contains the

well-conserved active site sequence Trp-Cys-Gly-Pro-Cys. Compared to thioredoxins from other sources, the algal thioredoxin Ch1 displays few sequence similarities with all the thioredoxins sequenced so far. Preliminary evidence indicates that Ch1 may be an h-type thioredoxin.

L15 ANSWER 7 OF 8 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 91:348492 SCISEARCH
THE GENUINE ARTICLE: FQ786
TITLE: CHARACTERIZATION AND PRIMARY STRUCTURE OF A 2ND THIOREDOXIN FROM THE GREEN-ALGA, CHLAMYDOMONAS-REINHARDTII
AUTHOR: DECOTTIGNIES P (Reprint); SCHMITTER J M; DUTKA S; JACQUOT J P; MIGINIACMASLOW M
CORPORATE SOURCE: UNIV PARIS 11, CNRS, PHYSIOL VEGETALE MOLEC LAB, BAT 430, F-91405 ORSAY, FRANCE (Reprint); ECOLE POLYTECH, BIOCHIM LAB, F-91128 PALAISEAU, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1991) Vol. 198, No. 2, pp. 505-512.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 45

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A second thioredoxin, Ch1, distinct from the one recently reported [Decottignies, P., Schmitter, J. M., Jacquot, J. P., Dutka, S., Picaud, A. & Gadal, P. (1990) Arch. Biochem. Biophys. 280, 112-121] has been purified from the green alga, Chlamydomonas reinhardtii, and its functional and structural properties investigated. Its activity in various enzymatic assays has been compared with the activities of different plant thioredoxins (Ch2 from C. reinhardtii and spinach m and f). Ch1 cannot serve as a substrate for Escherichia coli **thioredoxin reductase**, but can be reduced by spinach ferredoxin-**thioredoxin reductase**. It is less efficient than its spinach counterpart in the activation of corn leaf NADP-dependent malate dehydrogenase by light or dithiothreitol, and it only activates spinach fructose-1,6-bisphosphatase at very high concentrations. The complete primary structure of C. reinhardtii thioredoxin Ch1 was determined by automated Edman degradation of the intact protein and of peptides derived from trypsin, chymotrypsin and Staphylococcus **aureus** V8 protease digestions. When needed, peptide masses were verified by plasma desorption mass spectrometry. Ch1 consists of a polypeptide of 111 amino acids (11634 Da) and contains the well-conserved active site sequence Trp-Cys-Gly-Pro-Cys. Compared to thioredoxins from other sources, the algal thioredoxin Ch1 displays few sequence similarities with all the thioredoxins sequenced so far. Preliminary evidence indicates that Ch1 may be an h-type thioredoxin.

L15 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 5

ACCESSION NUMBER: 1981:564196 CAPLUS
DOCUMENT NUMBER: 95:164196
TITLE: Purification, characterization, and amino acid sequence of thioredoxin from Corynebacterium nephridii
AUTHOR(S): Meng, Mei; Hogenkamp, Harry P. C.
CORPORATE SOURCE: Med. Sch., Univ. Minnesota, Minneapolis, MN, 55455, USA
SOURCE: Journal of Biological Chemistry (1981), 256(17), 9174-82
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A thioredoxin was purified to homogeneity from cell-free exts. of C. nephridii using conventional techniques. This thioredoxin serves as the reducing substrate for yeast methionine sulfoxide reductase, for Escherichia coli **thioredoxin reductase**, and for Lactobacillus leichmannii ribonucleotide reductase. In contrast, thioredoxin from C. nephridii is unable to serve as a reducing substrate for ribonucleotide reductase from the same organism in the presence of either C. nephridii or E. coli **thioredoxin reductase**.
When reduced with dithiothreitol, thioredoxin from C. nephridii activates spinach chloroplast fructose-1,6-diphosphatase, NADP-malate dehydrogenase,

and phosphoribulokinase. The complete primary structure of the thioredoxin was detd. by automated Edman degrdn. and carboxypeptidase Y digestion of the intact protein and of peptides derived from Staphylococcus **aureus** protease and clostripain digestion and from CNBr cleavage. It consists of 105 amino acid residues. Comparison of this sequence with that of thioredoxin from E. coli reveals an amazing homol. between these 2 proteins. Almost half of the amino acid residues of the proteins are identical; the regions of homol. include the active site of thioredoxin and the regions which provide the interaction between the 2 domains of the mol.

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=>
=>
=> s thioredoxin reductase and (crystal or x-ray or nmr or model)
L16         160 FILE MEDLINE
L17         124 FILE CAPLUS
L18         176 FILE SCISEARCH
L19          33 FILE LIFESCI
L20         125 FILE BIOSIS
L21         115 FILE EMBASE

TOTAL FOR ALL FILES
L22         733 THIOREDOXIN REDUCTASE AND (CRYSTAL OR X-RAY OR NMR OR MODEL)

=> s l22 not 2001-2004/py
L23          94 FILE MEDLINE
L24          69 FILE CAPLUS
L25          91 FILE SCISEARCH
L26          23 FILE LIFESCI
L27          67 FILE BIOSIS
L28          64 FILE EMBASE

TOTAL FOR ALL FILES
L29         408 L22 NOT 2001-2004/PY

=> s l29 and (aureus or coli or thaliana)
L30          52 FILE MEDLINE
L31          31 FILE CAPLUS
L32          54 FILE SCISEARCH
L33          17 FILE LIFESCI
L34          33 FILE BIOSIS
L35          27 FILE EMBASE

TOTAL FOR ALL FILES
L36         214 L29 AND (AUREUS OR COLI OR THALIANA)

=> dup rem l36
PROCESSING COMPLETED FOR L36
L37           94 DUP REM L36 (120 DUPLICATES REMOVED)

=> d ti so au abs 1-94

L37  ANSWER 1 OF 94  SCISEARCH  COPYRIGHT (c) 2004 The Thomson Corporation.  on
     STN                                                    DUPLICATE 1
TI   Synthesis and characterization of selenotrisulfide-derivatives of lipoic
     acid and lipoamide
SO   PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
     AMERICA, (7 NOV 2000) Vol. 97, No. 23, pp. 12481-12486.
     Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC
     20418.
     ISSN: 0027-8424.
AU   Self W T (Reprint); Tsai L; Stadtman T C
AB      Thiol-containing compounds, such as glutathione and cysteine. react
     with selenite under specific conditions to form selenotrisulfides.
     Previous studies have focused on isolation and characterization of
     intermolecular selenotrisulfides. This study describes the preparation and
     characterization of intramolecular selenotrisulfide derivatives of lipoic
     acid and lipoamide. These derivatives, after separation from other
     reaction products by reverse-phase HPLC, exhibit an absorbance maximum at
     288 nm with an extinction coefficient of 1.500 M-1.cm(-1). The
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selenotrisulfide derivative of lipoic acid was significantly stable at or below pH 8.0 in contrast to several other previously studied selenotrisulfides. Mass spectral analysis of the lipoic acid and lipoamide derivatives confirmed both the expected molecular weights and also the presence of a single atom of selenium as revealed by its isotopic distribution. The selenotrisulfide derivative of lipoic acid was found to serve as an effective substrate for recombinant human **thioredoxin reductase** as well as native rat **thioredoxin reductase** in the presence of NADPH. Likewise, the lipoamide derivative was efficiently reduced by NADH-dependent bovine lipoamide dehydrogenase. The significant in vitro stability of these intramolecular selenotrisulfide derivatives of lipoic acid can serve as an important asset in the study of such selenium adducts as **model** selenium donor compounds for selenophosphate biosynthesis and as rate enhancement effecters in various redox reactions.

L37 ANSWER 2 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI Attachment of the N-terminal domain of Salmonella typhimurium AhpF to Escherichia **coli thioredoxin reductase** confers AhpC reductase activity but does not affect **thioredoxin reductase** activity

SO BIOCHEMISTRY, (1 AUG 2000) Vol. 39, No. 30, pp. 8859-8869.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0006-2960.

AU Reynolds C M; Poole L B (Reprint)

AB AhpF of Salmonella typhimurium, the flavoprotein reductase required for catalytic turnover of AhpC with hydroperoxide substrates in the alkyl hydroperoxide reductase system, is a 57 kDa protein with homology to **thioredoxin reductase** (TrR) from Escherichia **coli**. Like TrR, AhpF employs tightly bound FAD and redox-active disulfide center(s) in catalyzing electron transfer from reduced pyridine nucleotides to the disulfide bond of its protein substrate, Homology of AhpF to the smaller (35 kDa) TrR protein occurs in the C-terminal part of AhpF; a stretch of about 200 amino acids at the N-terminus of AhpF; contains an additional redox-active disulfide center and is required for catalysis of AhpC reduction. We have demonstrated that fusion of the N-terminal 207 amino acids of AhpF to full-length TrR results in a chimeric protein (Nt-TrR) with essentially the same catalytic efficiency (k(cat)/K-m) as AhpF in AhpC reductase assays; both k(cat) and the K-m for AhpC are decreased about 3-4-fold for Nt-TrR compared with AhpF. in addition, Nt-TrR retains essentially full TrR activity. Based on results from two mutants of Nt-TrR (C129,132S and C342,345S), AhpC reductase activity requires both centers while TrR activity requires only the C-terminal-most disulfide center in Nt-TrR. The high catalytic efficiency with which Nt-TrR can reduce thioredoxin implies that the attached N-terminal domain does not block access of thioredoxin to the TrR-derived Cys342-Cys345 center of Nt-TrR nor does it impede the putative conformational changes that this part of Nt-TrR is proposed to undergo during catalysis. These studies indicate that the C-terminal part of AhpF and bacterial TrR have very similar mechanistic properties. These findings also confirm that the N-terminal domain of AhpF plays a direct role in AhpC reduction.

L37 ANSWER 3 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI Identification and functional characterization of thioredoxin from Trypanosoma brucei brucei

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (17 MAR 2000) Vol. 275, No. 11, pp. 7547-7552.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.

AU Reckenfelderbaumer N; Ludemann H; Schmidt H; Steverding D; KrauthSiegel R L (Reprint)

AB Trypanosomes and Leishmania, the causative agents of several tropical diseases, lack the glultathione/glutathione reductase system but have trypanothione/ trypanothione reductase instead. The uniqueness of this thiol metabolism and the failure to detect **thioredoxin reductases** in these parasites have led to the suggestion that these protozoa lack a thioredoxin system. As presented here, this is not

the case. A gene encoding thioredoxin has been cloned from Trypanosoma brucei, the causative agent of African sleeping sickness. The single copy gene, which encodes a protein of 107 amino acid residues, is expressed in all developmental stages of the parasite. The deduced protein sequence is 56% identical with a putative thioredoxin revealed by the genome pro ject of Leishmania major. The relationship to other thioredoxins is low. T. brucei thioredoxin is unusual in having a calculated pi value of 8.5. The gene has been overexpressed in Escherichia **coli**. The recombinant protein is a substrate of human **thioredoxin reductase** with a K-m value of 6 mu M but is not reduced by trypanothione reductase, T. brucei thioredoxin catalyzes the reduction of insulin by dithioerythritol, and functions as an electron donor for T. brucei ribonucleotide reductase. The parasite protein is the first classical thioredoxin of the order Kinetoplastida characterized so far.

L37 ANSWER 4 OF 94 MEDLINE on STN DUPLICATE 2
TI A thioredoxin from the hyperthermophilic archaeon Methanococcus jannaschii has a glutaredoxin-like fold but thioredoxin-like activities.
SO Biochemistry, (2000 Jun 6) 39 (22) 6652-9.
Journal code: 0370623. ISSN: 0006-2960.
AU Lee D Y; Ahn B Y; Kim K S
AB A thioredoxin homologue (Mj0307) from the hyperthermophilic archaeon Methanococcus jannaschii (MjTRX) was cloned, produced in E. **coli** , and compared to the thioredoxin from E. **coli** (ETRX). The secondary structure profile of MjTRX obtained by **NMR** spectroscopy shows that it has four beta-sheets and three alpha-helices arranged in betaalphabetaalphabetabetaalpha, similar to that of glutaredoxin. However, MjTRX supports the growth of T7 bacteriophage in E. **coli** and is weakly reduced by the **thioredoxin reductase** from E. **coli**, indicating that MjTRX is functionally closer to a thioredoxin than a glutaredoxin. MjTRX has higher specific insulin reductase activity than ETRX and retained its full activity over 4 days at 95 degrees C, whereas ETRX lost its activity in 150 min. The standard state redox potential of MjTRX is about -277 mV, which is the lowest value thus far known among redox potentials of the thioredoxin superfamily. This indicates that the lower redox potential is necessary in keeping catalytic disulfide bonds reduced in the cytoplasm and in coping with oxidative stress in an anaerobic hyperthermophile.

L37 ANSWER 5 OF 94 MEDLINE on STN
TI AhpF can be dissected into two functional units: tandem repeats of two thioredoxin-like folds in the N-terminus mediate electron transfer from the **thioredoxin reductase**-like C-terminus to AhpC.
SO Biochemistry, (2000 Jun 6) 39 (22) 6602-15.
Journal code: 0370623. ISSN: 0006-2960.
AU Poole L B; Godzik A; Nayeem A; Schmitt J D
AB AhpF, the flavin-containing component of the Salmonella typhimurium alkyl hydroperoxide reductase system, catalyzes the NADH-dependent reduction of an active-site disulfide bond in the other component, AhpC, which in turn reduces hydroperoxide substrates. The amino acid sequence of the C-terminus of AhpF is 35% identical to that of **thioredoxin reductase** (TrR) from Escherichia **coli**. AhpF contains an additional 200-residue N-terminal domain possessing a second redox-active disulfide center also required for AhpC reduction. Our studies indicate that this N-terminus contains a tandem repeat of two thioredoxin (Tr)-like folds, the second of which contains the disulfide redox center. Structural and catalytic properties of independently expressed fragments of AhpF corresponding to the TrR-like C-terminus (F[208-521]) and the 2Tr-like N-terminal domain (F[1-202]) have been addressed. Enzymatic assays, reductive titrations, and circular dichroism studies of the fragments indicate that each folds properly and retains many functional properties. Electron transfer between F[208-521] and F[1-202] is, however, relatively slow (4 x 10(4) M(-)(1) s(-)(1) at 25 degrees C) and nonsaturable up to 100 microM F[1-202]. TrR is nearly as efficient at F[1-202] reduction as is F[208-521], although neither the latter fragment, nor intact AhpF, can reduce Tr. An engineered mutant AhpC substrate with a fluorophore attached via a disulfide bond has been used to demonstrate that only F[1-202], and not F[208-521], is capable of electron transfer to AhpC, thereby establishing the direct role this N-terminal domain plays in mediating electron transfer between the TrR-like part of AhpF and AhpC.

L37 ANSWER 6 OF 94 MEDLINE on STN
TI AhpF and other NADH:peroxiredoxin oxidoreductases, homologues of low Mr **thioredoxin reductase**.
SO European journal of biochemistry / FEBS, (2000 Oct) 267 (20) 6126-33.
Ref: 59
Journal code: 0107600. ISSN: 0014-2956.
AU Poole L B; Reynolds C M; Wood Z A; Karplus P A; Ellis H R; Li Calzi M
AB A group of bacterial flavoproteins related to **thioredoxin reductase** contain an additional approximately 200-amino-acid domain including a redox-active disulfide center at their N-termini. These flavoproteins, designated NADH:peroxiredoxin oxidoreductases, catalyze the pyridine-nucleotide-dependent reduction of cysteine-based peroxidases (e.g. Salmonella typhimurium AhpC, a member of the peroxiredoxin family) which in turn reduce H2O2 or organic hydroperoxides. These enzymes catalyze rapid electron transfer (kcat > 165 s-1) through one tightly bound FAD and two redox-active disulfide centers, with the N-terminal-most disulfide center acting as a redox mediator between the **thioredoxin-reductase**-like part of these proteins and the peroxiredoxin substrates. A chimeric protein with the first 207 amino acids of S. typhimurium AhpF attached to the N-terminus of Escherichia **coli thioredoxin reductase** exhibits very high NADPH:peroxiredoxin oxidoreductase and **thioredoxin reductase** activities. Catalytic turnover by NADH:peroxiredoxin oxidoreductases may involve major domain rotations, analogous to those proposed for bacterial **thioredoxin reductase**, and cycling of these enzymes between two electron-reduced (EH2) and four electron-reduced (EH4) redox states.

L37 ANSWER 7 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 3
TI **Thioredoxin reductase** - Two modes of catalysis have evolved
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (OCT 2000) Vol. 267, No. 20, pp. 6110-6117.
Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND.
ISSN: 0014-2956.
AU Williams C H (Reprint); Arscott L D; Muller S; Lennon B W; Ludwig M L; Wang P F; Veine D M; Becker K; Schirmer R H
AB **Thioredoxin reductase** (EC 1.6.4.5) is a widely distributed flavoprotein that catalyzes the NADPH-dependent reduction of thioredoxin. Thioredoxin plays several key roles in maintaining the redox environment of the cell. Like all members of the enzyme family that includes lipoamide dehydrogenase, glutathione reductase and mercuric reductase, **thioredoxin reductase** contains a redox active disulfide adjacent to the flavin ring. Evolution has produced two forms of **thioredoxin reductase**, a protein in prokaryotes, archaea and lower eukaryotes having a M-r of 35 000, and a protein in higher eukaryotes having a M-r of 55 000. Reducing equivalents are transferred from the apolar flavin binding site to the protein substrate by distinct mechanisms in the two forms of **thioredoxin reductase**. In the low M-r enzyme, interconversion between two conformations occurs twice in each catalytic cycle. After reduction of the disulfide by the flavin, the pyridine nucleotide domain must rotate with respect to the flavin domain in order to expose the nascent dithiol for reaction with thioredoxin; this motion repositions the pyridine ring adjacent to the flavin ring. In the high M-r enzyme, a third redox active group shuttles the reducing equivalent from the apolar active site to the protein surface. This group is a second redox active disulfide in **thioredoxin reductase** from Plasmodium falciparum and a selenenylsulfide in the mammalian enzyme. P. falciparum is the major causative agent of malaria and it is hoped that the chemical difference between the two high M-r forms may be exploited for drug design.

L37 ANSWER 8 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Structure and mechanism of mammalian **thioredoxin reductase**: The active site is a redox-active selenolthiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF

AMERICA, (23 MAY 2000) Vol. 97, No. 11, pp. 5854-5859.
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418.
ISSN: 0027-8424.

AU Zhong L W; Arner E S J; Holmgren A (Reprint)

AB Mammalian **thioredoxin reductases** (TrxR) are homodimers, homologous to glutathione reductase (CR), with an essential selenocysteine (SeCys) residue in an extension containing the conserved C-terminal sequence -Gly-Cys-SeCys-Gly, In the oxidized enzyme, we demonstrated two nonflavin redox centers by chemical modification and peptide sequencing: one was a disulfide within the sequence -Cys(59)-Val-Asn-Val-Gly-Cys(64), identical to the active site of CR; the other was a selenenylsulfide formed from Cys(497)-SeCys(498) and confirmed by mass spectrometry. In the NADPH reduced enzyme, these centers were present as a dithiol and a selenolthiol, respectively. Based on the structure of GR, we propose that in TrxR, the C-terminal Cys(497)-SeCys(498) residues Of one monomer are adjacent to the Cys(59) and Cys(64) residues of the second monomer, The reductive half-reaction of TrxR is similar to that of GR followed by exchange from the nascent Cys59 and Cys64 dithiol to the selenenylsulfide of the other subunit to generate the active-site selenolthiol. Characterization of recombinant mutant rat TrxR with SeCys(498) replaced by Cys having a 100-fold lower k(cat) for Trx reduction revealed the C-terminal redox center was present as a dithiol when the Cys(59)-Cys(64) was a disulfide, demonstrating that the selenium atom with its larger radius is critical for formation of the unique selenenylsulfide. Spectroscopic redox titrations with dithionite or NADPH were consistent with the structure **model**. Mechanisms of TrxR in reduction of Trx and hydroperoxides have been postulated and are compatible with known enzyme activities and the effects of inhibitors, like goldthioglucose and 1-chloro-2,4-dinitrobenzene.

L37 ANSWER 9 OF 94 MEDLINE on STN

TI Mammalian **thioredoxin reductase**: oxidation of the C-terminal cysteine/selenocysteine active site forms a thioselenide, and replacement of selenium with sulfur markedly reduces catalytic activity.

SO Proceedings of the National Academy of Sciences of the United States of America, (2000 Mar 14) 97 (6) 2521-6.
Journal code: 7505876. ISSN: 0027-8424.

AU Lee S R; Bar-Noy S; Kwon J; Levine R L; Stadtman T C; Rhee S G

AB Mammalian cytosolic **thioredoxin reductase** (TrxR) has a redox center, consisting of Cys(59)/Cys(64) adjacent to the flavin ring of FAD and another center consisting of Cys(497)/selenocysteine (SeCys)(498) near the C terminus. We now show that the C-terminal Cys(497)-SH/SeCys(498)-Se(-) of NADPH-reduced enzyme, after anaerobic dialysis, was converted to a thioselenide on incubation with excess oxidized Trx (TrxS(2)) or H(2)O(2). The Cys(59)-SH/Cys(64)-SH pair also was oxidized to a disulfide. At lower concentrations of TrxS(2), the Cys(59)-SH/Cys(64)-SH center was still converted to a disulfide, presumably by reduction of the thioselenide to Cys(497)-SH/SeCys(498)-Se(-). Specific alkylation of SeCys(498) completely blocked the TrxS(2)-induced oxidation of Cys(59)-SH/Cys(64)-SH, and the alkylated enzyme had negligible NADPH-disulfide oxidoreductase activity. The effect of replacing SeCys(498) with Cys was determined by using a mutant form of human placental TrxR1 expressed in Escherichia **coli**. The NADPH-disulfide oxidoreductase activity of the purified Cys(497)/Cys(498) mutant enzyme was 6% or 11% of that of wild-type rat liver TrxR1 with 5, 5'-dithiobis(2-nitrobenzoic acid) or TrxS(2), respectively, as substrate. Disulfide formation induced by excess TrxS(2) in the mutant form was 12% of that of the wild type. Thus, SeCys has a critical redox function during the catalytic cycle, which is performed poorly by Cys.

L37 ANSWER 10 OF 94 MEDLINE on STN DUPLICATE 4

TI Purification, crystallization and preliminary crystallographic data for rat cytosolic selenocysteine 498 to cysteine mutant **thioredoxin reductase**.

SO Acta crystallographica. Section D, Biological crystallography, (2000 Sep) 56 (Pt 9) 1191-3.
Journal code: 9305878. ISSN: 0907-4449.

AU Zhong L; Persson K; Sandalova T; Schneider G; Holmgren A

AB Mammalian cytosolic **thioredoxin reductase** is a homodimer of 55 kDa subunit containing an essential penultimate

selenocysteine residue. An active analogue of the rat enzyme in which cysteine replaces selenocysteine has been expressed in Escherichia **coli** cells at high levels and purified to homogeneity. The pure enzyme contains one FAD per subunit and shows spectral properties identical to that of the wild-type **thioredoxin reductase** . The isolated enzyme in its oxidized and reduced forms or the enzyme complexed with NADP(+) was crystallized by the hanging-drop vapour-diffusion method. The diffraction pattern extends to 3 A resolution. The **crystals** are monoclinic, space group P2(1), with unit-cell parameters a = 78.9, b = 140.5, c = 170.8 A, alpha = 94.6 degrees. There are three dimeric molecules in the asymmetric unit.

L37 ANSWER 11 OF 94 MEDLINE on STN DUPLICATE 5
TI Twists in catalysis: alternating conformations of Escherichia **coli** **thioredoxin reductase**.
SO Science, (2000 Aug 18) 289 (5482) 1190-4.
Journal code: 0404511. ISSN: 0036-8075.
AU Lennon B W; Williams C H Jr; Ludwig M L
AB In **thioredoxin reductase** (TrxR) from Escherichia **coli**, cycles of reduction and reoxidation of the flavin adenine dinucleotide (FAD) cofactor depend on rate-limiting rearrangements of the FAD and NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) domains. We describe the structure of the flavin-reducing conformation of E. **coli** TrxR at a resolution of 3.0 angstroms. The orientation of the two domains permits reduction of FAD by NADPH and oxidation of the enzyme dithiol by the protein substrate, thioredoxin. The alternate conformation, described by Kuriyan and co-workers, permits internal transfer of reducing equivalents from reduced FAD to the active-site disulfide. Comparison of these structures demonstrates that switching between the two conformations involves a "ball-and-socket" motion in which the pyridine nucleotide-binding domain rotates by 67 degrees.

L37 ANSWER 12 OF 94 MEDLINE on STN DUPLICATE 6
TI Transfer of electrons across the cytoplasmic membrane by DsbD, a membrane protein involved in thiol-disulphide exchange and protein folding in the bacterial periplasm.
SO Molecular microbiology, (2000 Mar) 35 (5) 1099-109.
Journal code: 8712028. ISSN: 0950-382X.
AU Chung J; Chen T; Missiakas D
AB Reduction of non-native protein disulphides in the periplasm of Escherichia **coli** is catalysed by three enzymes, DsbC, DsbG and DsbE, each of which harbours a catalytic Cys-X-X-Cys dithiol motif. This dithiol motif requires continuous reduction for activity. Genetic evidence suggests that the source of periplasmic reducing power resides within the cytoplasm, provided by thioredoxin (trxA) and **thioredoxin reductase** (trxB). Cytoplasmic electrons donated by thioredoxin are thought to be transferred into the periplasm via the DsbD membrane protein. To understand the molecular nature of electron transfer, we have analysed the membrane topology of DsbD. DsbD is exported by an N-terminal signal peptide. The N- and C-terminal domains are positioned in the periplasmic space, connected by eight transmembrane segments. Electron transfer was shown to require five cysteine sulphydryl of DsbD. Trans complementation of mutant DsbD molecules revealed intermolecular electron transfer. We discuss a **model** whereby the membrane-embedded disulphides of DsbD accept electrons from cytoplasmic thioredoxin and transfer them to the C-terminal periplasmic dithiol motif of DsbD.

L37 ANSWER 13 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Selenium metabolism in zebrafish: multiplicity of selenoprotein genes and expression of a protein containing 17 selenocysteine residues
SO GENES TO CELLS, (DEC 2000) Vol. 5, No. 12, pp. 1049-1060.
Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND.
ISSN: 1356-9597.
AU Kryukov G V; Gladyshev V N (Reprint)
AB Background: Fish are an important source of selenium in human nutrition and the zebrafish is a potentially useful **model** organism for the study of selenium metabolism and its role in biology and medicine.

Selenium is present in vertebrate proteins in the form of selenocysteine (Sec), the 21st natural amino acid in protein which is encoded by UGA.

Results: We report here the detection of 18 zebrafish genes for Sec-containing proteins. We found two zebrafish orthologs of human SelT, glutathione peroxidase 1 and glutathione peroxidase 4, and single orthologs of several other selenoproteins. In addition, new zebrafish selenoproteins were identified that were distant homologues of SelP, SelT and SelW, but their direct orthologs in other species are not known. This multiplicity of selenoprotein genes appeared to result from gene and genome duplications, followed by the retention of new selenoprotein genes. We found a zebrafish selenoprotein P gene (designated zSelPa) that contained two Sec insertion sequence (SECIS) elements and encoded a protein containing 17 Sec residues, the largest number of Sec residues found in any known protein. In contrast, a second SelP gene (designated zSelPb) was also identified that contained one SECIS element and encoded a protein with a single Sec. We found that zSelPa could be expressed and secreted by mammalian cells.

Conclusions: The occurrence of zSelPa and zSelPb suggested that the function of the N-terminal domain of mammalian SelP proteins may be separated from that of the C-terminal Sec-rich sequence: the N-terminal domain containing the UxxC motif is likely involved in oxidoreduction, whereas the C-terminal portion of the protein may function in selenium transport or storage. Our data also suggest that the utilization of Sec is more common in zebrafish than in previously characterized species, including mammals.

L37 ANSWER 14 OF 94 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 7

TI Selenium in biology: Facts and medical perspectives.

SO Biological Chemistry, (Sept.-Oct., 2000) Vol. 381, No. 9-10, pp. 849-864. print.
ISSN: 1431-6730.

AU Koehrle, Josef [Reprint author]; Brigelius-Flohe, Regina; Boeck, August; Gaertner, Roland; Meyer, Ortwin; Flohe, Leopold

AB Several decades after the discovery of selenium as an essential trace element in vertebrates approximately 20 eukaryotic and more than 15 prokaryotic selenoproteins containing the 21st proteinogenic amino acid, selenocysteine, have been identified, partially characterized or cloned from several species. Many of these proteins are involved in redox reactions with selenocysteine acting as an essential component of the catalytic cycle. Enzyme activities have been assigned to the glutathione peroxidase family, to the **thioredoxin reductases**, which were recently identified as selenoproteins, to the iodothyronine deiodinases, which metabolize thyroid hormones, and to the selenophosphate synthetase 2, which is involved in selenoprotein biosynthesis. Prokaryotic selenoproteins catalyze redox reactions and formation of selenoethers in (stress-induced) metabolism and energy production of E. **coli**, of the clostridial cluster XI and of other prokaryotes. Apart from the specific and complex biosynthesis of selenocysteine, selenium also reversibly binds to proteins, is incorporated into selenomethionine in bacteria, yeast and higher plants, or posttranslationally modifies a catalytically essential cysteine residue of CO dehydrogenase. Expression of individual eukaryotic selenoproteins exhibits high tissue specificity, depends on selenium availability, in some cases is regulated by hormones, and if impaired contributes to several pathological conditions. Disturbance of selenoprotein expression or function is associated with deficiency syndromes (Keshan and Kashin-Beck disease), might contribute to tumorigenesis and atherosclerosis, is altered in several bacterial and viral infections, and leads to infertility in male rodents.

L37 ANSWER 15 OF 94 MEDLINE on STN

TI Antioxidant function of thioredoxin and glutaredoxin systems.

SO Antioxidants & redox signalling, (2000 Winter) 2 (4) 811-20. Ref: 58
Journal code: 100888899. ISSN: 1523-0864.

AU Holmgren A

AB Selenium is an essential trace element with known antioxidant properties. Cytosolic **thioredoxin reductase** from mammalian cells is a dimeric flavin enzyme comprising a glutathione reductase-like equivalent elongated with 16 residues including the conserved carboxy-terminal sequence, Gly-Cys-SeCys-Gly, where SeCys is

selenocysteine. Replacement of the SeCys residue by Cys in rat cytosolic **thioredoxin reductase** using site-directed mutagenesis and expression in Escherichia **coli** resulted in a functional mutant enzyme having about one percent activity with thioredoxin as a substrate through a major loss of Kcat and a shift in the pH optimum from 7 to 9. The truncated enzyme expected in selenium deficiency by the UGA mRNA codon for SeCys acting as a stop codon was also expressed. This enzyme lacking the carboxy-terminal SeCys-Gly dipeptide contained FAD but was inactive because the SeCys selenol is in the active site. These results show that selenium is essential for the activity of **thioredoxin reductase**, explaining why this trace element is required for cell proliferation by effects on thioredoxin-dependent control of the intracellular redox state, ribonucleotide reductase production of deoxyribonucleotides, or activation of transcription factors. The selenazol drug ebselen (2-phenyl-1,2 benzisoselenazol-3 (2H)-one) is a known glutathione (GSH) peroxidase mimic with antioxidant properties. The hydrogen peroxide reductase activity of human **thioredoxin reductase** was stimulated 15-fold by 2 microM ebselen. Glutaredoxins protect against oxidative stress by catalyzing reduction of protein mixed disulfides with GSH. The mechanism of glutaredoxins as efficient general GSH-mixed disulfide oxidoreductases may protect proteins from inactivation as well as play a major role in general redox signaling.

L37 ANSWER 16 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI **NMR** structures of thioredoxin m from the green alga Chlamydomonas reinhardtii

SO PROTEINS-STRUCTURE FUNCTION AND GENETICS, (15 NOV 2000) Vol. 41, No. 3, pp. 334-349.
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012.
ISSN: 0887-3585.

AU Lancelin J M (Reprint); Guilhaudis L; Krimm I; Blackledge M J; Marion D; Jacquot J P

AB Chloroplast thioredoxin in from the green alga Chlamydomomas reinhardtii is very efficiently reduced in vitro and in vivo in the presence of photoreduced ferredoxin and a ferredoxin dependent ferredoxin-**thioredoxin reductase**, Once reduced, thioredoxin m has the capability to quickly activate the NADP malate dehydrogenase (EC 1.1.1.82) a regulatory enzyme involved in an energy-dependent assimilation of carbon dioxide in C4 plants. This activation is the result of the reduction of two disulfide bridges by thioredoxin m, that are located at the N- and C-terminii of the NADP malate dehydrogenase. The molecular structure of thioredoxin m was solved using **NMR** and compared to other known thioredoxins, Thioredoxin m belongs to the prokaryotic type of thioredoxin, which is divergent from the eukaryotic-type thioredoxins also represented in plants by the h (cytosolic) and f (chloroplastic) types of thioredoxins, The dynamics of the molecule have been assessed using N-15 relaxation data and are found to correlate well with regions of disorder found in the calculated MMR ensemble. The results obtained provide a novel basis to interpret the thioredoxin dependence of the activation of chloroplast NADP-malate dehydrogenase, The specific catalytic mechanism that takes place in the active site of thioredoxins is also discussed on the basis of the recent new understanding and especially in the light of the dual general acid-base catalysis exerted on the two cysteines of the redox active site. It is proposed that the two cysteines of the redox active site may insulate each other from solvent attack by specific packing of invariable hydrophobic amino acids. (C) 2000 Wiley-Liss, Inc.

L37 ANSWER 17 OF 94 MEDLINE on STN DUPLICATE 8

TI Crystallization and preliminary **X-ray** analysis of the catalytic core of the alkylhydroperoxide reductase component AhpF from Escherichia **coli**.

SO Acta crystallographica. Section D, Biological crystallography, (2000 Jan) 56 (Pt 1) 92-4.
Journal code: 9305878. ISSN: 0907-4449.

AU Bieger B; Essen L O

AB Alkylhydroperoxide reductases (AhpR, E.C. 1.6.4.x) are essential for the oxygen tolerance of aerobic organisms, converting otherwise toxic hydroperoxides of lipids or nucleic acids to their corresponding alcohols.

The AhpF component (521 amino-acid residues, 56.2 kDa) belongs to the family of pyridine nucleotide-disulfide oxidoreductases and channels electrons from NAD(P)H via a series of disulfides towards the AhpC component, which finally reduces the hydro-peroxide substrates. **Crystals** of the proteolytically truncated AhpF component (residues Asn208-Ala521) of the alkyl hydroperoxide reductase from Escherichia **coli** were grown under oxidizing conditions. The **crystals** belong to space group P3(2)21, with unit-cell parameters a = 60.4, c = 171.8 A. **X-ray** diffraction data were collected to 1.9 A resolution using synchrotron radiation. A molecular-replacement solution was found using the structure of **thioredoxin reductase** from Arabidopsis **thaliana** as a search **model**.

L37 ANSWER 18 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI Structural determinants for the efficient and specific interaction of thioredoxin with 2-oxoacid dehydrogenase complexes

SO APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, (JUL-SEP 2000) Vol. 88, No. 1-3, pp. 77-96.
Publisher: HUMANA PRESS INC, 999 RIVERVIEW DRIVE SUITE 208, TOTOWA, NJ 07512.
ISSN: 0273-2289.

AU Raddatz G; Kruft V; Bunik V (Reprint)

AB Specificity and efficiency of thioredoxin action upon the 2-oxoacid dehydrogenase complexes are studied by using a number of thioredoxins and complexes. Bacterial and mammalian pyruvate and 2-oxoglutarate dehydrogenase systems display similar row of preference to thioredoxins that may result from thioredoxin binding to the homologous or common dihydrolipoamide dehydrogenase components of the complexes. The most sensitive to thioredoxin is the complex whose component exhibits the highest sequence similarity to eukaryotic **thioredoxin reductase**. Hence, thioredoxin binding to the complexes may be related to that in the **thioredoxin reductase**, a dihydrolipoamide dehydrogenase homolog. The highest potency of mitochondrial thioredoxin to affect the mitochondrial complexes is revealed. A 96-100% conservation of the mitochondrial thioredoxin structure is shown within the four known sequences and the N-terminus of the pig heart protein determined. Eleven thioredoxins tested biochemically are analyzed by multiple sequence alignment and homology modeling. Their effects correlate with the residues at the contact between the alpha 3/3(10) and alpha 1 helices, the length of the al helix and charges in the alpha 2-beta 3 and beta 4-beta 5 linkers. Polarization of the thioredoxin molecule and its active site surroundings are characterized. Thioredoxins with a highly polarized surface around the essential disulfide bridge (mitochondrial, pea f, and Arabidopsis **thaliana** h3) show low crossreactivity as compared to the species with a decreased polarization of this area (e.g., from Escherichia **coli**). The strongest polarization of the whole molecule results in the highest magnitude of the electrostatic dipole vector of mitochondrial thioredoxin. Thioredoxins with the dipole orientation similar to that of the latter have the affinities for the 2-oxoacid dehydrogenase complexes, proportional to the dipole magnitudes. Thioredoxin with an opposite dipole orientation shows no effect. Activating and inhibitory thioredoxin disulfides are distinguished by the charges of the residues 13/14 (alpha 1 helix), 51 (alpha 2-beta 3 linker), and 83/85 (beta 4-beta 5 linker), changing the dipole direction. The results show that the thioredoxin-target interplay may be controlled by the long-range interactions between the electrostatic dipole vectors of the proteins and the degree of their interface polarization.

L37 ANSWER 19 OF 94 MEDLINE on STN DUPLICATE 9

TI Importance of redox potential for the in vivo function of the cytoplasmic disulfide reductant thioredoxin from Escherichia **coli**.

SO Journal of biological chemistry, (1999 Sep 3) 274 (36) 25254-9.
Journal code: 2985121R. ISSN: 0021-9258.

AU Mossner E; Huber-Wunderlich M; Rietsch A; Beckwith J; Glockshuber R; Aslund F

AB The thioredoxin superfamily consists of enzymes that catalyze the reduction, formation, and isomerization of disulfide bonds and exert their activity through a redox active disulfide in a Cys-Xaa(1)-Xaa(2)-Cys

motif. The individual members of the family differ strongly in their intrinsic redox potentials. However, the role of the different redox potentials for the in vivo function of these enzymes is essentially unknown. To address the question of in vivo importance of redox potential for the most reducing member of the enzyme family, thioredoxin, we have employed a set of active site variants of thioredoxin with increased redox potentials (-270 to -195 mV) for functional studies in the cytoplasm of Escherichia **coli**. The variants proved to be efficient substrates of **thioredoxin reductase**, providing a basis for an in vivo characterization of NADPH-dependent reductive processes catalyzed by the thioredoxin variants. The reduction of sulfate and methionine sulfoxide, as well as the isomerization of periplasmic disulfide bonds by DsbC, which all depend on thioredoxin as catalyst in the E. **coli** cytoplasm, proved to correlate well with the intrinsic redox potentials of the variants in complementation assays. The same correlation could be established in vitro by using the thioredoxin-catalyzed reduction of lipoic acid by NADPH as a **model** reaction. We propose that the rate of direct reduction of substrates by thioredoxin, which largely depends on the redox potential of thioredoxin, is the most important parameter for the in vivo function of thioredoxin, as recycling of reduced thioredoxin through NADPH and **thioredoxin reductase** is not rate-limiting for its catalytic cycle.

L37 ANSWER 20 OF 94 MEDLINE on STN DUPLICATE 10
TI G33D mutant thioredoxin primarily affects the kinetics of reaction with **thioredoxin reductase**. Probing the structure of the mutant protein.
SO Biochemistry, (1999 Nov 23) 38 (47) 15508-13.
Journal code: 0370623. ISSN: 0006-2960.
AU Lin T Y
AB Escherichia **coli** thioredoxin is a redox-active protein. A mutant protein with an aspartic acid substitution for the largely conserved glycine at position 33 (G33D) in the active site of thioredoxin has been generated to study the effects of a negatively charged residue in the active site of the protein. Despite the close proximity of the negative-charged Asp to the redox active cysteines, the effective concentration of the cysteines does not deviate significantly from that of the wild-type protein. The redox potential (E(o)') measured by the equilibrium between NADPH and the mutant thioredoxin is also close to that of the wild-type. Kinetic measurements of the reaction between thioredoxin and **thioredoxin reductase** show that G33D mutant and the wild-type proteins have identical kcat values. However, the Km for G33D mutant is approximately 10-fold higher than that for the wild-type protein. In vivo assay of the growth of E. **coli** strain carrying wild-type or G33D mutant thioredoxin on methionine sulfoxide indicates that the G33D mutant protein is a slower electron donor for methionine sulfoxide reductase. Structural stability of the oxidized protein is not altered by the G33D substitution, as illustrated by the same unfolding free energies studied by urea. The substitution does not show significant change of the near UV and far-UV circular dichroic (CD) and the fluorescence spectra for either the reduced or the oxidized protein. Therefore, the global structure of the G33D protein is not changed. However, the surface of the active site has been altered locally by G33D substitution, which accounts for the above kinetically poor behaviors. A **model** of G33D structure is constructed based on these studies.

L37 ANSWER 21 OF 94 MEDLINE on STN DUPLICATE 11
TI **Crystal** structure of reduced **thioredoxin reductase** from Escherichia **coli**: structural flexibility in the isoalloxazine ring of the flavin adenine dinucleotide cofactor.
SO Protein science : a publication of the Protein Society, (1999 Nov) 8 (11) 2366-79.
Journal code: 9211750. ISSN: 0961-8368.
AU Lennon B W; Williams C H Jr; Ludwig M L
AB Catalysis by **thioredoxin reductase** (TrxR) from Escherichia **coli** requires alternation between two domain arrangements. One of these conformations has been observed by **X -ray** crystallography (Waksman G, Krishna TSR, Williams CH Jr, Kuriyan J, 1994, J Mol Biol 236:800-816). This form of TrxR, denoted FO, permits the reaction of enzyme-bound reduced FAD with a redox-active

disulfide on TrxR. As part of an investigation of conformational changes and intermediates in catalysis by TrxR, an **X-ray** structure of the FO form of TrxR with both the FAD and active site disulfide reduced has been determined. Reduction after crystallization resulted in significant local conformation changes. The isoalloxazine ring of the FAD cofactor, which is essentially planar in the oxidized enzyme, assumes a 34 degree "butterfly" bend about the N(5)-N(10) axis in reduced TrxR. Theoretical calculations reported by others predict ring bending of 15-28 degrees for reduced isoalloxazines protonated at N(1). The large bending in reduced TrxR is attributed in part to steric interactions between the isoalloxazine ring and the sulfur of Cys138, formed by reduction of the active site disulfide, and is accompanied by changes in the positions and interactions of several of the ribityl side-chain atoms of FAD. The bending angle in reduced TrxR is larger than that for any flavoprotein in the Protein Data Bank. Distributions of bending angles in published oxidized and reduced flavoenzyme structures are different from those found in studies of free flavins, indicating that the protein environment has a significant effect on bending.

L37 ANSWER 22 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with **thioredoxin reductase**

SO CARCINOGENESIS, (SEP 1999) Vol. 20, No. 9, pp. 1657-1666.
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0143-3334.

AU Ganther H E (Reprint)

AB Numerous studies in animal **models** and more recent studies in humans have demonstrated cancer chemopreventive effects with Se. There is extensive evidence that monomethylated forms of Se are critical metabolites for chemopreventive effects of Se. Induction of apoptosis in transformed cells is an important chemopreventive mechanism. Apoptosis can be triggered by micromolar levels of monomethylated forms of Se independent of DNA damage and in cells having a null p53 phenotype, Cell cycle protein kinase cdk2 and protein kinase C are strongly inhibited by various forms of Se, Inhibitory mechanisms involving modification of cysteine residues in proteins by Se have been proposed that involve formation of Se adducts of the selenotrisulfide (S-Se-S) or selenenylsulfide (S-Se) type or catalysis of disulfide formation. Selenium may facilitate reactions of protein cysteine residues by the transient formation of more reactive S-Se intermediates. A novel chemopreventive mechanism is proposed involving Se catalysis of reversible cysteine/disulfide transformations that occur in a number of redox-regulated proteins, including transcription factors. A time-limited activation mechanism for such proteins, with deactivation facilitated by Se, would allow normalization of critical cellular processes in the early stages of transformation. There is uncertainty at the present time regarding the role of selenoproteins in chemoprevention **model** systems where supranutritional levels of Se are employed. Mammalian **thioredoxin reductase** is one selenoprotein that shows increased activity with Se supplementation in the nutritional to supranutritional range. Enhanced thioredoxin reduction could have beneficial effects in oxidative stress, but possible adverse effects are considered. Other functions of **thioredoxin reductase** may be relevant to cell signaling pathways. The functional status of the thioredoxin/**thioredoxin reductase** system during in vivo chemoprevention with Se has not been established. Some in vitro studies have shown inhibitory effects of Se on the thioredoxin system correlated with growth inhibition by Se. A potential inactivating mechanism for **thioredoxin reductase** or other selenoenzymes involving formation of a stable deselenide form resistant to reduction is discussed, New aspects of Se biochemistry and possible functions of new selenoproteins in chemoprevention are described.

L37 ANSWER 23 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI The structure of adrenodoxin reductase of mitochondrial P450 systems: Electron transfer for steroid biosynthesis

SO JOURNAL OF MOLECULAR BIOLOGY, (18 JUN 1999) Vol. 289, No. 4, pp. 981-990.
Publisher: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND.
ISSN: 0022-2836.

AU Ziegler G A; Vonrhein C; Hanukoglu I; Schulz G E (Reprint)

AB Adrenodoxin reductase is a monomeric 51 kDa flavoenzyme that is involved in the biosynthesis of all steroid hormones. The structure of the native bovine enzyme was determined at 2.8 Angstrom resolution, and the structure of the respective recombinant enzyme at 1.7 Angstrom resolution. Adrenodoxin reductase receives a two-electron package from NADPH and converts it to two single electrons that are transferred via adrenodoxin to all mitochondrial cytochromes P450. The structure suggests how the observed flavin semiquinone is stabilized. A striking feature is the asymmetric charge distribution, which most likely controls the approach of the electron carrier adrenodoxin. A **model** for the interaction is proposed. Adrenodoxin reductase shows clear sequence homology to half a dozen proteins identified in genome analysis projects, but neither sequence nor structural homology to established, functionally related electron transferases. Yet, the structure revealed a relationship to the disulfide oxidoreductases, permitting the assignment of the NADP-binding site. (C) 1999 Academic Press.

L37 ANSWER 24 OF 94 MEDLINE on STN DUPLICATE 12

TI Minithioredoxin: a folded and functional peptide fragment of thioredoxin.

SO Biochemical and biophysical research communications, (1999 Aug 11) 261 (3) 676-81.
Journal code: 0372516. ISSN: 0006-291X.

AU Ghoshal A K

AB A peptide fragment comprising the first 83 residues from the N-terminus of E. **coli** thioredoxin is purified by hydroxylamine cleavage of the intact protein. At physiological pH, the secondary and tertiary structure contents of the peptide are 70 and 35%, respectively, compared to the intact protein. Peptide 83 is able to display dual biological functions of thioredoxin, namely, a substrate for the enzyme E. **coli thioredoxin-reductase** and a processivity factor of T7 DNA polymerase. At present, peptide 83 represents the minimum functional and folding unit of thioredoxin. The highly conserved residue Phe 81 appears to play an important role in the folding of peptide 83, as judged from the packing analysis. Peptide 83 also mimics a particular kinetic folding intermediate of thioredoxin in terms of spectral properties and may serve as an equilibrium peptide **model** for the former.

L37 ANSWER 25 OF 94 MEDLINE on STN

TI Activation of active-site cysteine residues in the peroxiredoxin-type tryparedoxin peroxidase of Crithidia fasciculata.

SO European journal of biochemistry / FEBS, (1999 Sep) 264 (2) 516-24.
Journal code: 0107600. ISSN: 0014-2956.

AU Montemartini M; Kalisz H M; Hecht H J; Steinert P; Flohe L

AB Tryparedoxin peroxidase (TXNPx), recently identified as the hydroperoxide-detoxifying enzyme of trypanosomatidae [Nogoceke, E., Gommel, D. U., Kiess, M., Kalisz, H. M. & Flohe, L. (1997) Biol. Chem. 378, 827-836], is a member of the peroxiredoxin family and is characterized by two VCP motifs. Based on a consensus sequence of TXNPx and peroxiredoxin-type peroxidases, eight TXNPx variants were designed, heterologously expressed in Escherichia **coli**, checked for alpha-helix content by CD and kinetically analysed. The variant Q164E was fully active, C52S, W87D and R128E were inactive and C173S, W87H, W177E and W177H showed reduced activity. Wild-type TXNPx and Q164E exhibit ping-pong kinetics with infinite maximum velocities, whereas saturation kinetics were observed with C173S and W177E. The data comply with a mechanism in which C52, primarily activated by R128 and possibly by W87, is first oxidized by hydroperoxide to a sulfenic acid derivative. C173, supported by W177, then forms an intersubunit disulfide bridge with C52. If C173 is exchanged with a redox-inactive residue (Ser) or is insufficiently activated, the redox shuttle remains restricted to C52. The shift in the kinetic pattern and decrease in specific activity of C173S and W177E may result from a limited accessibility of the oxidized C52 to tryparedoxin, which in the oxidized wild-type TXNPx presumably attacks the C173 sulfur of the disulfide bridge. The proposed mechanism of action of TXNPx is consistent with that deduced for the homologous thioredoxin peroxidase of yeast [Chae, H. Z., Uhm, T. B. & Rhee, S. G. (1994) Proc. Natl Acad. Sci. USA 91, 7022-7026] and is supported by molecular modelling based on the structure of the human peroxiredoxin 'hORF6' [Choi, H.-J., Kang, S. W. Yang, C.-H., Rhee, S. G. & Ryu, S.-E.

(1998) Nat. Struct. Biol. 5, 400-406].

L37 ANSWER 26 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Preparation and assay of mammalian thioredoxin and **thioredoxin reductase**
SO METHODS IN ENZYMOLOGY, (MAR 1999) Vol. 300, pp. 226-239.
Publisher: ACADEMIC PRESS INC, 525 B STREET, SUITE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0076-6879.
AU Arner E S J (Reprint); Zhong L W; Holmgren A

L37 ANSWER 27 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Microinjected glutathione reductase **crystals** as indicators of the redox status in living cells
SO FEBS LETTERS, (26 MAR 1999) Vol. 447, No. 2-3, pp. 135-138.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0014-5793.
AU Keese M A; Saffrich R; Dandekar T; Becker K; Schirmer R H (Reprint)
AB The flavoenzyme glutathione reductase catalyses electron transfer reactions between two major intracellular redox buffers, namely the NADPH/NADP(+) couple and the 2 glutathione/glutathione disulfide couple, On this account, microcrystals of the enzyme were tested as redox probes of intracellular compartments. For introducing protein **crystals** into human fibroblasts, different methods (microinjection, particle bombardment and optical tweezers) were explored and compared. When glutathione reductase **crystals** are present in a cytosolic environment, the transition of the yellow E-OX form to the orange-red 2-electron reduced charge transfer form, EH2, is observed. Taking into account the midpoint potential of the E-OX/ EH2 couple, the redox potential of the cytosol was found to be <-270 mV at pH 7.4 and 37 degrees C. As a general conclusion, competent proteins in crystalline - that is signal-amplifying form are promising probes for studying intracellular events. (C) 1999 Federation of European Biochemical Societies.

L37 ANSWER 28 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Disulfide bond formation and folding of plant peroxidases expressed as inclusion body protein in Escherichia **coli thioredoxin reductase** negative strains
SO PROTEIN EXPRESSION AND PURIFICATION, (FEB 1999) Vol. 15, No. 1, pp. 77-82.
Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 1046-5928.
AU Teilum K; Ostergaard L; Welinder K G (Reprint)
AB Escherichia **coli** is widely used for the production of proteins, which are of interest in structure and function studies. The folding yield of inclusion body protein is, however, generally low (a few percent) for proteins such as the plant and fungal peroxidases, which contain four disulfide bonds, two Ca2+ ions, and a heme group. We have studied the expression yield and folding efficiency of (i) a novel Arabidopsis **thaliana** peroxidase, ATP N; and (ii) barley grain peroxidase, BP 1. The expression yield ranges from 0 to 60 mu g/ml of cell culture depending on the peroxidase gene and the vector/host combination. The choice off. **coli** strain in particular affects the yield of active peroxidase obtained in the folding step. Thus, the yield of active ATP N peroxidase can be increased 50-fold by using **thioredoxin reductase** negative strains, which facilitate the formation of disulfide bonds in inclusion body protein. (C) 1999 Academic Press.

L37 ANSWER 29 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Role of electrostatic interactions on the affinity of thioredoxin for target proteins - Recognition of chloroplast fructose-1,6-bisphosphatase by mutant Escherichia **coli** thioredoxins
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (26 JUN 1998) Vol. 273, No. 26, pp. 16273-16280.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.

ISSN: 0021-9258.

AU MoraGarcia S; RodriguezSuarez R; Wolosiuk R A (Reprint)

AB Chloroplast thioredoxin-f functions efficiently in the light-dependent activation of chloroplast fructose-1,6-bisphosphatase by reducing a specific disulfide bond located at the negatively charged domain of the enzyme. Around the nucleophile cysteine of the active site (-W-C-G-P-C-), chloroplast thioredoxin-f shows lower density of negative charges than the inefficient modulator Escherichia **coli** thioredoxin, To examine the contribution of long range electrostatic interactions to the thiol/disulfide exchange between protein-disulfide oxidoreductases and target proteins, we constructed three variants off. **coli** thioredoxin in which an acidic (Glu-30) and a neutral residue (Leu-94) were replaced by lysines, After purification to homogeneity, the reduction of the unique disulfide bond by NADPH via NADP-**thioredoxin reductase** proceeded at similar rates for all variants. However, the conversion of cysteine residues back to cystine depended on the target protein. Insulin and difluoresceinthiocarbamyl-insulin oxidized the sulfhydryl groups of E30K and E30K/L94K mutants more effectively than those of wild type and L94K counterparts, Moreover, the affinity of E30g, L94R, and E30K/L94K E. **coli** thioredoxin for chloroplast fructose-1,6-bisphosphatase (A(0.5) = 9, 7, and 3 mu M, respectively) increased with the number of positive charges, and was higher than wild type thioredoxin (A(0.5)= 33 mu M), though still lower than that of thioredoxin-f (A(0.5) = 0.9 mu M). We also demonstrated that shielding of electrostatic interactions with high salt concentrations not only brings the A(0.5) for all bacterial variants to a limiting value of similar to 9 mu M but also increases the A,, of chloroplast thioredoxin-f, While negatively charged chloroplast fructose-1,6-bisphosphatase (pI = 4.9) readily interacted with mutant thioredoxins, the reduction rate of rapeseed napin (pI = 11.2) diminished with the number of novel lysine residues. These findings suggest that the electrostatic interactions between thioredoxin and (some of) its target proteins controls the formation of the binary noncovalent complex needed for the subsequent thiol/disulfide exchange.

L37 ANSWER 30 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI sigma(R), an RNA polymerase sigma factor that modulates expression of the thioredoxin system in response to oxidative stress in Streptomyces coelicolor A3(2)

SO EMBO JOURNAL, (1 OCT 1998) Vol. 17, No. 19, pp. 5776-5782.
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0261-4189.

AU Paget M S B (Reprint); Kang J G; Roe J H; Buttner M J

AB We have identified an RNA polymerase sigma factor, sigma(R), that is part of a system that senses and responds to thiol oxidation in the Gram-positive, antibiotic-producing bacterium Streptomyces coelicolor A3(2), Deletion of the gene (sigR) encoding sigma(R) caused sensitivity to the thiol-specific oxidant diamide and to the redox cycling compounds menadione and plumbagin, This correlated with reduced levels of disulfide reductase activity and an inability to induce this activity on exposure to diamide, The trxBA operon, encoding **thioredoxin reductase** and thioredoxin, was found to be under the direct control of sigma(R). trxBA is transcribed from two promoters, trxBp1 and trxBp2, separated by 5-6 bp, trxBp1 is transiently induced at least 50-fold in response to diamide treatment in a sigR-dependent manner. Purified sigma(R) directed transcription from trxBp1 in vitro, indicating that trxBp1 is a target for sigma(R). Transcription of sigR itself initiates at two promoters, sigRp1 and sigRp2, which are separated by 173 bp. The sigRp2 transcript was undetectable in a sigR-null mutant, and purified sigma(R) could direct transcription from sigRp2 in vitro, indicating that sigR is positively autoregulated. transcription from sigRp2 was also transiently induced (70-fold) following treatment with diamide. We propose a **model** in which sigma(R) induces expression of the thioredoxin system in response to cytoplasmic disulfide bond formation. Upon reestablishment of normal thiol levels, sigma(R) activity is switched off, resulting in down-regulation of trxBA and sigR. We present evidence that the sigma(R) system also functions in the actinomycete pathogen Mycobacterium tuberculosis.

L37 ANSWER 31 OF 94 MEDLINE on STN DUPLICATE 13

TI Disulfide bond formation in the Escherichia **coli** cytoplasm: an in vivo role reversal for the thioredoxins.
SO EMBO journal, (1998 Oct 1) 17 (19) 5543-50.
Journal code: 8208664. ISSN: 0261-4189.
AU Stewart E J; Aslund F; Beckwith J
AB Cytoplasmic proteins do not generally contain structural disulfide bonds, although certain cytoplasmic enzymes form such bonds as part of their catalytic cycles. The disulfide bonds in these latter enzymes are reduced in Escherichia **coli** by two systems; the thioredoxin pathway and the glutathione/glutaredoxin pathway. However, structural disulfide bonds can form in proteins in the cytoplasm when the gene (trxB) for the enzyme **thioredoxin reductase** is inactivated by mutation. This disulfide bond formation can be detected by assessing the state of the normally periplasmic enzyme alkaline phosphatase (AP) when it is localized to the cytoplasm. Here we show that the formation of disulfide bonds in cytoplasmic AP in the trxB mutant is dependent on the presence of two thioredoxins in the cell, thioredoxins 1 and 2, the products of the genes trxA and trxC, respectively. Our evidence supports a **model** in which the oxidized forms of these thioredoxins directly catalyze disulfide bond formation in cytoplasmic AP, a reversal of their normal role. In addition, we show that the recently discovered thioredoxin 2 can perform many of the roles of thioredoxin 1 in vivo, and thus is able to reduce certain essential cytoplasmic enzymes. Our results suggest that the three most effective cytoplasmic disulfide-reducing proteins are thioredoxin 1, thioredoxin 2 and glutaredoxin 1; expression of any one of these is sufficient to support aerobic growth. Our results help to explain how the reducing environment in the cytoplasm is maintained so that disulfide bonds do not normally occur.

L37 ANSWER 32 OF 94 MEDLINE on STN DUPLICATE 14
TI Protein hydration in solution: experimental observation by **x-ray** and neutron scattering.
SO Proceedings of the National Academy of Sciences of the United States of America, (1998 Mar 3) 95 (5) 2267-72.
Journal code: 7505876. ISSN: 0027-8424.
AU Svergun D I; Richard S; Koch M H; Sayers Z; Kuprin S; Zaccai G
AB The structure of the protein-solvent interface is the subject of controversy in theoretical studies and requires direct experimental characterization. Three proteins with known atomic resolution **crystal** structure (lysozyme, Escherichia **coli** **thioredoxin reductase**, and protein R1 of E. **coli** ribonucleotide reductase) were investigated in parallel by **x-ray** and neutron scattering in H2O and D2O solutions. The analysis of the protein-solvent interface is based on the significantly different contrasts for the protein and for the hydration shell. The results point to the existence of a first hydration shell with an average density approximately 10% larger than that of the bulk solvent in the conditions studied. Comparisons with the results of other studies suggest that this may be a general property of aqueous interfaces.

L37 ANSWER 33 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Sirohaem sulfite reductase and other proteins encoded by genes at the dsr locus of Chromatium vinosum are involved in the oxidation of intracellular sulfur
SO MICROBIOLOGY-UK, (JUL 1998) Vol. 144, Part 7, pp. 1881-1894.
Publisher: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING RG7 1AE, BERKS, ENGLAND.
ISSN: 1350-0872.
AU Pott A S; Dahl C (Reprint)
AB The sequence of the dsr gene region of the phototrophic sulfur bacterium Chromatium vinosum D (DSMZ 180(T)) was determined to clarify the in vivo role of 'reverse' sirohaem sulfite reductase. The dsrAB genes encoding dissimilatory sulfite reductase are part of a gene cluster, dsrABEFHCMK, that encodes four small, soluble proteins (DsrE, DsrF, DsrH and DsrC), a transmembrane protein (DsrM) with similarity to haem-ij-binding polypeptides and a soluble protein (DsrK) resembling [4Fe-4S]-cluster-containing heterodisulfide reductase from methanogenic archaea. Northern hybridizations showed that expression of the dsr genes is increased by the presence of reduced sulfur compounds. The dsr genes are not only transcribed from a putative promoter upstream of dsrA but

primary transcripts originating from (a) transcription start site(s) downstream of dsrB are also formed. Polar insertion mutations immediately upstream of dsrA, and in dsrB, dsrH and dsrM, led to an inability of the cells to oxidize intracellularly stored sulfur. The capability of the mutants to oxidize sulfide, thiosulfate and sulfite under photolithoautotrophic conditions was unaltered. Photoorganoheterotrophic growth was also unaffected. 'Reverse' sulfite reductase and DsrEFHCMK are, therefore, not essential for oxidation of sulfide or thiosulfate, but are obligatory for sulfur oxidation. These results, together with the finding that the sulfur globules of C. vinosum are located in the extracytoplasmic space whilst the dsr gene products appear to be either cytoplasmic or membrane-bound led to the proposal of new **models** for the pathway of sulfur oxidation in this phototrophic sulfur bacterium.

L37 ANSWER 34 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI Formation and properties of mixed disulfides between **thioredoxin reductase** from Escherichia **coli** and thioredoxin: Evidence that cysteine-138 functions to initiate dithiol-disulfide interchange and to accept the reducing equivalent from reduced flavin

SO PROTEIN SCIENCE, (JUN 1998) Vol. 7, No. 6, pp. 1441-1450. Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211. ISSN: 0961-8368.

AU Veine D M; Mulrooney S B; Wang P F; Williams C H (Reprint)

AB Mutation of one of the cysteine residues in the redox active disulfide of **thioredoxin reductase** from Escherichia **coli** results in C135S with Cys(138) remaining or C138S with Cys(135) remaining. The expression system for the genes encoding **thioredoxin reductase**, wild-type enzyme, C135S, and C138S has been re-engineered to allow for greater yields of protein. Wild-type enzyme and C135S were found to be as previously reported, whereas discrepancies were detected in the characteristics of C138S. It was shown that the original C138S was a heterogeneous mixture containing C138S and wild-type enzyme and that enzyme obtained from the new expression system is the correct species. C138S obtained from the new expression system having 0.1% activity and 7% flavin fluorescence of wild-type enzyme was used in this study. Reductive titrations show that, as expected, only 1 mol of sodium dithionite/mol of FAD is required to reduce C138S, The remaining thiol in C135S and C138S has been reacted with 5,5'-dithiobis-(2-nitrobenzoic acid) to form mixed disulfides. The half time of the reaction was <5 s for Cys(138) in C135S and approximately 300 s for Cys(135) in C138S showing that Cys(138) is much more reactive. The resulting mixed disulfides have been reacted with Cys(32) in C35S mutant thioredoxin to form stable, covalent adducts C138S-C35S and C135S-C35S, The half times show that Cys(138) is approximately fourfold more susceptible to attack by the nucleophile. These results suggest that Cys(138) may be the thiol initiating dithiol-disulfide interchange between **thioredoxin reductase** and thioredoxin.

L37 ANSWER 35 OF 94 MEDLINE on STN DUPLICATE 15

TI **Thioredoxin reductase** from Escherichia **coli**: evidence of restriction to a single conformation upon formation of a crosslink between engineered cysteines.

SO Protein science : a publication of the Protein Society, (1998 Feb) 7 (2) 369-75. Journal code: 9211750. ISSN: 0961-8368.

AU Veine D M; Ohnishi K; Williams C H Jr

AB **Thioredoxin reductase** is a flavoprotein which catalyzes the reduction of the small protein thioredoxin by NADPH. It contains a redox active disulfide and an FAD in each subunit of its dimeric structure. Each subunit is further divided into two domains, the FAD and the pyridine nucleotide binding domains. The orientation of the two domains determined from the **crystal** structure and the flow of electrons determined from mechanistic studies suggest that **thioredoxin reductase** requires a large conformational change to carry out catalysis (Williams CH Jr, 1995, FASEB J 9:1267-1276). The constituent amino acids of an ion pair, E48/R130, between the FAD and pyridine nucleotide binding domains, were mutagenized to cysteines to form E48C,R130C (CC mutant). Formation of a stable bridge between these cysteines was expected to restrict the enzyme largely in the conformation

observed in the **crystal** structure. Crosslinking with the bifunctional reagent N,N,1,2 phenylenedimaleimide, spanning 4-9 A, resulted in a >95 % decrease in **thioredoxin reductase** and transhydrogenase activity. SDS-PAGE confirmed that the crosslink in the CC-mutant was intramolecular. Dithionite titration showed an uptake of electrons as in wild-type enzyme, but anaerobic reduction of the flavin with NADPH was found to be impaired. This indicates that the crosslinked enzyme is in the conformation where the flavin and the active site disulfide are in close proximity but the flavin and pyridinium rings are too far apart for effective electron transfer. The evidence is consistent with the hypothesis that **thioredoxin reductase** requires a conformational change to complete catalysis.

L37 ANSWER 36 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI Nitroreductase reactions of Arabidopsis **thaliana** **thioredoxin reductase**

SO BIOCHIMICA ET BIOPHYSICA ACTA-BIOENERGETICS, (7 SEP 1998) Vol. 1366, No. 3, pp. 275-283.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0005-2728.

AU Miskiniene V; Sarlauskas J; Jacquot J P; Cenas N (Reprint)

AB Arabidopsis **thaliana** NADPH:**thioredoxin** **reductase** (TR, EC 1.6.4.5) catalyzed redox cycling of aromatic nitrocompounds, including the explosives 2,4,6-trinitrotoluene and tetryl, and the herbicide 3,5-dinitro-o-cresol. The yield of nitro anion radicals was equal to 70-90%, Redox cycling of tetryl was accompanied by formation of N-methylpicramide. Bimolecular rate constants of nitroaromatic reduction (k(cat)/K-m) and reaction catalytic constants (k(cat)) increased upon an increase in oxidant single-electron reduction potential (E-7(1)). Using the compounds with an unknown E-7(1) value, the reactivity of TR increased parallelly to the increase in reactivity of ferredoxin:NADP(+) reductase of Anabaena PCC 7119 (EC 1.18.1.2). This indicated that the main factor determining reactivity of nitroaromatics towards TR was their energetics of single-electron reduction. Incubation of reduced TR in the presence of tetryl or 2,4-dinitrochlorobenzene resulted in a loss of **thioredoxin reductase** activity, most probably due to modification of reduced catalytic disulfide, whereas nitroreductase reaction rates were unchanged. This means that on the analogy of quinone reduction by TR (D. Bironaite, Z. Anusevicius, J.-P. Jacquot, N. Cenas, Biochim. Biophys. Acta 1383 (1998) 82-92), FAD and not catalytic disulfide of TR was responsible for the reduction of nitroaromatics. Tetryl, 2,4,6-trinitrotoluene and thioredoxin increased the FAD fluorescence intensity of TR. This finding suggests that nitroaromatics may bind close to the thioredoxin-binding site at the catalytic disulfide domain of TR, and induce a conformational change of enzymes (S.B. Mulrooney, C.H. Williams Jr., Protein Sci. 6 (1997) 2188-2195). Our data indicate that certain nitroaromatic herbicides, explosives and other classes of xenobiotics may interfere with the reduction of thioredoxin by plant TR, and confer prooxidant properties to this antioxidant enzyme. (C) 1998 Elsevier Science B.V. All rights reserved.

L37 ANSWER 37 OF 94 MEDLINE on STN DUPLICATE 16

TI The single mutation Trp35-->Ala in the 35-40 redox site of Chlamydomonas reinhardtii thioredoxin h affects its biochemical activity and the pH dependence of C36-C39 1H-13C **NMR**.

SO European journal of biochemistry / FEBS, (1998 Jul 1) 255 (1) 185-95.
Journal code: 0107600. ISSN: 0014-2956.

AU Krimm I; Lemaire S; Ruelland E; Miginiac-Maslow M; Jaquot J P; Hirasawa M; Knaff D B; Lancelin J M

AB The role of the invariant Trp residue at the redox site of thioredoxins was investigated by site-directed mutagenesis of a Chlamydomonas reinhardtii thioredoxin h. Though being still redox active with NADPH-**thioredoxin reductase** and chemical substrates [dithiothreitol and 5,5'-dithio-bis(2-nitrobenzoic acid)] the Trp35-->Ala-mutated protein completely lost the capacity to activate the thiol-regulated NADPH-dependent malate dehydrogenase. However, it was able to activate a mutant malate dehydrogenase where only the most exposed disulfide was retained. The pH dependence of the redox-site Cys beta 1H/13C-**NMR** frequencies of the wild-type and mutated proteins, in

both the reduced and oxidised states, were compared over the pH range 5.8-10. The mutation does not affect the conserved buried Asp30, which titrates with a pKa of 7.5 in the oxidised proteins in agreement with previous studies. However, for the reduced forms of the proteins, the pH dependence of resonances of both Cys was strongly affected by the mutation. In the case of the wild-type thioredoxin, two apparent pKa values were found around 7.0 and 9.5 and could be assigned to the titration of Cys36 and Cys39 thiol, respectively, similar to the case of Escherichia **coli** thioredoxin. For the mutated thioredoxin a single pKa was found around 8.3. This result can be interpreted as a single pKa of either Cys36 or Cys39 or both. While the mutation clearly affects ionisations, the measured redox potentials of the active-site Cys pair are not significantly affected by the Trp35-->Ala mutation. Possible roles of an aromatic side chain on the reactivity of the catalytic Cys residues in thioredoxins are proposed.

L37 ANSWER 38 OF 94 MEDLINE on STN DUPLICATE 17
TI Interaction of quinones with Arabidopsis **thaliana** **thioredoxin reductase**.
SO Biochimica et biophysica acta, (1998 Mar 3) 1383 (1) 82-92. Journal code: 0217513. ISSN: 0006-3002.
AU Bironaite D; Anusevicius Z; Jacquot J P; Cenas N
AB In view of the ubiquitous role of the thioredoxin/**thioredoxin reductase** (TRX/TR) system in living cells, the interaction of Arabidopsis **thaliana** NADPH-**thioredoxin reductase** (EC 1.6.4.5) with quinones, an important class of redox cycling and alkylating xenobiotics, was studied. The steady-state reactions of A. **thaliana** TR with thioredoxin (TRX) and reaction product NADP+ inhibition patterns were in agreement with a proposed **model** of E. coli enzyme (B.W. Lennon, C.H. Williams, Jr., Biochemistry, vol. 35 (1996), pp. 4704-4712), that involved enzyme cycling between four- and two-electron reduced forms with FAD being reduced. Quinone reduction by TR proceeded via a mixed single- and two-electron transfer, the percentage of single-electron flux being equal to 12-16%. Bimolecular rate constants of quinone reduction (kcat/km) and reaction catalytic constants (kcat) increased upon an increase in quinone single-electron reduction potential. E(1)7. In several cases, the kcat of quinone reduction exceeded kcat of TRX reduction, suggesting that quinones intercepted electron flux from TR to TRX. Incubation of reduced TR with alkylating quinones resulted in a rapid loss of TRX-reductase activity, while quinone reduction rate was unchanged. In TRX-reductase and quinone reductase reactions of TR, NADP+ exhibited different inhibition patterns. These data point out that FAD and not the catalytic disulfide of TR is responsible for quinone reduction, and that quinones may oxidize FADH2 before it reduces catalytic disulfide. Most probably, quinones may oxidize the two-electron reduced form of TR, and the enzyme may cycle between two-electron reduced and oxidized forms in this reaction. The relatively high rate of quinone reduction by A. **thaliana thioredoxin reductase** accompanied by their redox cycling, confers pro-oxidant properties to this antioxidant enzyme. These factors make plant TR an attractive target for redox active and alkylating pesticide action.

L37 ANSWER 39 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Interaction of quinones with Arabidopsis **thaliana** **thioredoxin reductase**
SO BIOCHIMICA ET BIOPHYSICA ACTA-PROTEIN STRUCTURE AND MOLECULAR ENZYMOLOGY, (3 MAR 1998) Vol. 1383, No. 1, pp. 82-92.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0167-4838.
AU Bironaite D; Anusevicius Z; Jacquot J P; Cenas N (Reprint)
AB In view of the ubiquitous role of the thioredoxin/thioredodin reductase (TRX/TR) system in living cells, the interaction of Arabidopsis **thaliana** NADPH-**thioredoxin reductase** (EC 1.6.4.5) with quinones, an important class of redox cycling and allylating xenobiotics, was studied, The steady-state reactions of A. **thaliana** TR with thioredoxin (TRX) and reaction product NADP(+) inhibition patterns were in agreement with a proposed **model** of E. **coli** enzyme (B.W. Lennon, C.H. Williams, Jr., Biochemistry,

vol. 35 (1996), pp. 4704-4712), that involved enzyme cycling between four-and two-electron reduced forms with FAD being reduced. Quinone reduction by TR proceeded via a mixed single-and two-electron transfer, the percentage of single-electron flux being equal to 12-16%. Bimolecular rate constants of quinone reduction (k(cat)/k(m)) and reaction catalytic constants (k(cat)) increased upon an increase in quinone single-electron reduction potential, E-1(7). In several cases, the k(cat) of quinone reduction exceeded k(cat) of TRX reduction, suggesting that quinones intercepted electron flux from TR to TRX. Incubation of reduced TR with alkylating quinones resulted in a rapid loss of TRX-reductase activity, while quinone reduction rate was unchanged. In TRX-reductase and quinone reductase reactions of TR, NADP(+) exhibited different inhibition patterns. These data point out that FAD and mt the catalytic disulfide of TR is responsible for quinone reduction, and that quinones may oxidize FADH(2) before it reduces catalytic disulfide. Most probably, quinones may oxidize the two-electron reduced form of TR, and the enzyme may cycle between two-electron reduced and oxidized forms in this reaction, The relatively high rate of quinone reduction by A. **thaliana** **thioredoxin reductase** accompanied bq-their redox cycling, confers pro-oxidant properties to this antioxidant-enzyme. These factors make plant TR an attractive target for redox active and alkylating pesticide action. (C) 1998 Elsevier Science B.V.

L37 ANSWER 40 OF 94 MEDLINE on STN
TI Redox potentials of glutaredoxins and other thiol-disulfide oxidoreductases of the thioredoxin superfamily determined by direct protein-protein redox equilibria.
SO Journal of biological chemistry, (1997 Dec 5) 272 (49) 30780-6. Journal code: 2985121R. ISSN: 0021-9258.
AU Aslund F; Berndt K D; Holmgren A
AB Glutaredoxins belong to the thioredoxin superfamily of structurally similar thiol-disulfide oxidoreductases catalyzing thiol-disulfide exchange reactions via reversible oxidation of two active-site cysteine residues separated by two amino acids (CX1X2C). Standard state redox potential (E degrees ') values for glutaredoxins are presently unknown, and use of glutathione/glutathione disulfide (GSH/GSSG) redox buffers for determining E degrees ' resulted in variable levels of GSH-mixed disulfides. To overcome this complication, we have used reverse-phase high performance liquid chromatography to separate and quantify the oxidized and reduced forms present in the thiol-disulfide exchange reaction at equilibrium after mixing one oxidized and one reduced protein. This allowed for direct and quantitative pair-wise comparisons of the reducing capacities of the proteins and mutant forms. Equilibrium constants from pair-wise reaction with thioredoxin or its P34H mutant, which have accurately determined E degrees ' values from their redox equilibrium with NADPH catalyzed by **thioredoxin** **reductase**, allowed for transformation into standard state values. Using this new procedure, the standard state redox potentials for the Escherichia **coli** glutaredoxins 1 and 3, which contain identical active site sequences CPYC, were found to be E degrees ' = -233 and -198 mV, respectively. These values were confirmed independently by using the thermodynamic linkage between the stability of the disulfide bond and the stability of the protein to denaturation. Comparison of calculated E degrees ' values from a number of proteins ranging from -270 mV for E. **coli** Trx to -124 mV for DsbA obtained using this method with those determined using glutathione redox buffers provides independent confirmation of the standard state redox potential of glutathione as -240 mV. Determining redox potentials through direct protein-protein equilibria is of general interest as it overcomes errors in determining redox potentials calculated from large equilibrium constants with the strongly reducing NADPH or by accumulating mixed disulfides with GSH.

L37 ANSWER 41 OF 94 MEDLINE on STN DUPLICATE 18
TI General acid/base catalysis in the active site of Escherichia **coli** thioredoxin.
SO Biochemistry, (1997 Dec 16) 36 (50) 15810-6. Journal code: 0370623. ISSN: 0006-2960.
AU Chivers P T; Raines R T
AB Enzymic catalysts of thiol:disulfide oxidoreduction contain two cysteine residues in their active sites. Another common residue is an aspartate (or glutamate), the role of which has been unclear. Escherichia

coli thioredoxin (Trx) is the best characterized thiol:disulfide oxidoreductase, and in Trx these three active-site residues are Cys32, Cys35, and Asp26. Structural analyses had indicated that the carboxylate of Asp26 is positioned properly for the deprotonation of the thiol of Cys35, which would facilitate its attack on Cys32 in enzyme-substrate mixed disulfides. Here, Asp26 of Trx was replaced with isologous asparagine and leucine residues. D26N Trx and D26L Trx are reduced and oxidized more slowly than is wild-type Trx during catalysis by E. **coli thioredoxin reductase**. Stopped-flow spectroscopy demonstrated that the cleavage of the mixed disulfide between Trx and a substrate is slower in the D26N and D26L enzymes. Buffers increase the rate of mixed disulfide cleavage in these variants but not in wild-type Trx. These results indicate that Asp26 serves as an acid/base in the oxidation/reduction reactions catalyzed by Trx. Specifically, Asp26 protonates (during substrate oxidation) or deprotonates (during substrate reduction) the thiol of Cys35. A similar role is likely filled by the analogous aspartate (or glutamate) residue in protein disulfide isomerase, DsbA, and other thiol:disulfide oxidoreductases. Moreover, these results provide the first evidence for general acid/base catalysis in a thiol:disulfide interchange reaction.

L37 ANSWER 42 OF 94 MEDLINE on STN
TI The role of the thioredoxin and glutaredoxin pathways in reducing protein disulfide bonds in the Escherichia **coli** cytoplasm.
SO Journal of biological chemistry, (1997 Jun 20) 272 (25) 15661-7.
Journal code: 2985121R. ISSN: 0021-9258.
AU Prinz W A; Aslund F; Holmgren A; Beckwith J
AB In Escherichia **coli**, two pathways use NADPH to reduce disulfide bonds that form in some cytoplasmic enzymes during catalysis: the thioredoxin system, which consists of **thioredoxin reductase** and thioredoxin, and the glutaredoxin system, composed of glutathione reductase, glutathione, and three glutaredoxins. These systems may also reduce disulfide bonds which form spontaneously in cytoplasmic proteins when E. **coli** is grown aerobically. We have investigated the role of both systems in determining the thiol-disulfide balance in the cytoplasm by determining the ability of protein disulfide bonds to form in mutants missing components of these systems. We find that both the thioredoxin and glutaredoxin systems contribute to reducing disulfide bonds in cytoplasmic proteins. In addition, these systems can partially substitute for each other in vivo since double mutants missing parts of both systems generally allow substantially more disulfide bond formation than mutants missing components of just one system. Some of these double mutants were found to require the addition of a disulfide reductant to the medium to grow well aerobically. Thus, E. **coli** requires either a functional thioredoxin or glutaredoxin system to reduce disulfide bonds which appear after each catalytic cycle in the essential enzyme ribonucleotide reductase and perhaps to reduce non-native disulfide bonds in cytoplasmic proteins. Our results suggest the existence of a novel thioredoxin in E. **coli**.

L37 ANSWER 43 OF 94 MEDLINE on STN
TI Reduction of the periplasmic disulfide bond isomerase, DsbC, occurs by passage of electrons from cytoplasmic thioredoxin.
SO Journal of bacteriology, (1997 Nov) 179 (21) 6602-8.
Journal code: 2985120R. ISSN: 0021-9193.
AU Rietsch A; Bessette P; Georgiou G; Beckwith J
AB The Escherichia **coli** periplasmic protein DsbC is active both in vivo and in vitro as a protein disulfide isomerase. For DsbC to attack incorrectly formed disulfide bonds in substrate proteins, its two active-site cysteines should be in the reduced form. Here we present evidence that, in wild-type cells, these two cysteines are reduced. Further, we show that a pathway involving the cytoplasmic proteins **thioredoxin reductase** and thioredoxin and the cytoplasmic membrane protein DsbD is responsible for the reduction of these cysteines. Thus, reducing potential is passed from cytoplasmic electron donors through the cytoplasmic membrane to DsbC. This pathway does not appear to utilize the cytoplasmic glutathione-glutaredoxin pathway. The redox state of the active-site cysteines of DsbC correlates quite closely with its ability to assist in the folding of proteins with multiple disulfide bonds. Analysis of the activity of mutant forms of DsbC in which either or both of these cysteines have been altered further

supports the role of DsbC as a disulfide bond isomerase.

L37 ANSWER 44 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI The mechanism of **thioredoxin reductase** from human placenta is similar to the mechanisms of lipoamide dehydrogenase and glutathione reductase and is distinct from the mechanism of **thioredoxin reductase** from Escherichia **coli**

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (15 APR 1997) Vol. 94, No. 8, pp. 3621-3626.
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418.
ISSN: 0027-8424.

AU Arscott L D; Gromer S; Schirmer R H; Becker K; Williams C H (Reprint)

AB **Thioredoxin reductase**, lipoamide dehydrogenase, and glutathione reductase are members of the pyridine nucleotide-disulfide oxidoreductase family of dimeric flavoenzymes. The mechanisms and structures of lipoamide dehydrogenase and glutathione reductase are alike irrespective of the source (subunit M-r approximate to 55,000). Although the mechanism and structure of **thioredoxin reductase** from Escherichia **coli** are distinct (M-r approximate to 35,000), this enzyme must be placed in the same family because there are significant amino acid sequence similarities with the other two enzymes, the presence of a redox-active disulfide, and the substrate specificities. **Thioredoxin reductase** from higher eukaryotes on the other hand has a M-r of approximate to 55,000 [Luthman, M. & Holmgren, A. (1982) Biochemistry 21, 6628-6633; Gasdaska, P. Y., Gasdaska, J. R., Cochran, S. & Powis, G. (1995) FEBS Lett 373, 5-9; Gladyshev, V. N., Jeang, K. T. & Stadtman, T.C. (1996) Proc. Natl. Acad. Sci. USA 93, 6146-6151]. Thus, the evolution of this family is highly unusual. The mechanism of **thioredoxin reductase** from higher eukaryotes is not known. As reported here, **thioredoxin reductase** from human placenta reacts with only a single molecule of NADPH, which leads to a stable intermediate similar to that observed in titrations of lipoamide dehydrogenase or glutathione reductase. Titration of **thioredoxin reductase** from human placenta with dithionite takes place in two spectral phases: formation of a thiolate-flavin charge transfer complex followed by reduction of the flavin, just as with lipoamide dehydrogenase or glutathione reductase. The first phase requires more than one equivalent of dithionite. This suggests that the penultimate selenocysteine [Tamura, T. & Stadtman, T.C. (1996) Proc. Natl. Acad. Sci. USA 93, 1006-1011] is in redox communication with the active site disulfide/dithiol. Nitrosoureas of the carmustine type inhibit only the NADPH reduced form of human **thioredoxin reductase**. These compounds are widely used as cytostatic agents, so this enzyme should be studied as a target in cancer chemotherapy. In conclusion, three lines of evidence indicate that the mechanism of human **thioredoxin reductase** is like the mechanisms of lipoamide dehydrogenase and glutathione reductase and differs fundamentally from the mechanism of E. **coli thioredoxin reductase**.

L37 ANSWER 45 OF 94 MEDLINE on STN DUPLICATE 19

TI Effects of buried charged groups on cysteine thiol ionization and reactivity in Escherichia **coli** thioredoxin: structural and functional characterization of mutants of Asp 26 and Lys 57.

SO Biochemistry, (1997 Mar 4) 36 (9) 2622-36.
Journal code: 0370623. ISSN: 0006-2960.

AU Dyson H J; Jeng M F; Tennant L L; Slaby I; Lindell M; Cui D S; Kuprin S; Holmgren A

AB To investigate the role of Asp 26 and Lys 57, two conserved, buried residues, in the redox mechanism of Escherichia **coli** thioredoxin (Trx), three mutant proteins, Asp 26 --> Ala (D26A), Lys 57 --> Met (K57M), and the double mutant D26A/K57M, were prepared, replacing the charged amino acids with hydrophobic residues with similar sizes. Both the oxidized (Trx-S2) and reduced [Trx-(SH)2] forms of the mutant thioredoxins are fully folded and similar in overall structure to the wild-type protein (wt). The structure of the active site hydrophobic surface is unchanged by the mutation of Asp 26 and Lys 57, since DNA polymerase activity in the 1:1 complex of the T7 gene 5 protein and mutant Trx-(SH)2 shows similar Kd values (approximately 5 nM) for both mutants

and wt. In contrast, redox reactions involving thioredoxin as a catalyst of the reduction of disulfides or oxidation of dithiols are strongly affected by the mutations. In the reaction of Trx-S2 with **thioredoxin reductase** at pH 8.0, the kcat/Km value for the D26A mutant is decreased by a factor of 10 from that of wt, while the value for the D26A/K57M mutant is reduced 40-fold. The activity of Trx-(SH)2 as a protein disulfide reductase was measured with insulin, using fluorescence to detect oxidation of thioredoxin. At 15 degrees C and pH 8.0, both the D26A and K57M mutants showed 5--10-fold decreases in rates of reaction compared to those of the wild type, and the pH-rate profiles for the mutants were shifted 1 (K57M) and 2 (D26A) units to higher pH compared with the wt curve. **NMR** measurements for the three mutant proteins indicate that the proteins have the same global fold as that of the wild type, although changes in the chemical shifts of a number of resonances indicate local structural changes in the active site region. The resonances of oxidized D26A and D26A/K57M are pH-independent between pH 6.0 and 10.0, confirming the identification of the active site group titrating with a pKa of 7.5 in wt Trx-S2 as Asp 26. A profound change in the pKa of Asp 26, from 7.5 in the wild type to 9.4 in the mutant, is observed for K57M Trx-S2. The pH-dependent behavior of the resonances is affected in all mutant Trx-(SH)2 proteins. A single pKa shifted to higher values is observed on both the Cys 32 and Cys 35 Cbeta resonances. Ultraviolet absorbance measurements (A240) as a function of pH for wt Trx-(SH)2 demonstrate that the cysteine thiols titrate with apparent pK(a)s of about 7.1 and 9.9. The mutant proteins each show a single transition in the A240 measurements, with a midpoint at pH 7.8-8.0, consistent with the **NMR** results. The change in absorbance at 240 nm with increasing pH indicates that the number of thiols titrating in each mutant is greater than one but less than two. It is clear that both thiol pK(a)s have been significantly shifted by the mutations. The Cys 32 pKa is moved from 7.1 in wt to 7.8-8.0 in the mutants. The value of the Cys 35 pKa either is indistinguishable from that of Cys 32, thus accounting for more than one thiol titrating in the UV absorbance measurements or else is shifted to much higher pHs (> 10) where its transition is masked in both UV and **NMR** measurements by the effects of ionization of the tyrosine residues and unfolding of the protein. Our results strongly suggest that the buried Asp 26 carboxyl and Lys 57 epsilon-amino groups significantly affect the pK(a)s of the active site thiols, particularly that of the exposed low-pKa thiol Cys 32, thereby enhancing the rates of thiol-disulfide reactions at physiological pH.

L37 ANSWER 46 OF 94 MEDLINE on STN DUPLICATE 20

TI Evidence for two conformational states of **thioredoxin reductase** from Escherichia **coli**: use of intrinsic and extrinsic quenchers of flavin fluorescence as probes to observe domain rotation.

SO Protein science : a publication of the Protein Society, (1997 Oct) 6 (10) 2188-95.
Journal code: 9211750. ISSN: 0961-8368.

AU Mulrooney S B; Williams C H Jr

AB **Thioredoxin reductase** (TrxR) from Escherichia **coli** consists of two globular domains connected by a two-stranded beta sheet: an FAD domain and a pyridine nucleotide binding domain. The latter domain contains the redox-active disulfide composed of Cys 135 and Cys 138. TrxR is proposed to undergo a conformational change whereby the two domains rotate 66 degrees relative to each other (Waksman G, Krishna TSR, Williams CH Jr, Kuriyan J, 1994, J Mol Biol 236:800-816), placing either redox active disulfide (FO conformation) or the NADPH binding site (FR conformation) adjacent to the flavin. This domain rotation **model** was investigated by using a Cys 138 Ser active-site mutant. The flavin fluorescence of this mutant is only 7% that of wild-type TrxR, presumably due to the proximity of Ser 138 to the flavin in the FO conformation. Reaction of the remaining active-site thiol, Cys 135, with phenylmercuric acetate (PMA) causes a 9.5-fold increase in fluorescence. Titration of the PMA-treated mutant with the nonreducing NADP(H) analogue, 3-aminopyridine adenine dinucleotide phosphate (AADP+), results in significant quenching of the flavin fluorescence, which demonstrates binding adjacent to the FAD, as predicted for the FR conformation. Wild-type TrxR, with or without PMA treatment, shows similar quenching by AADP+, indicating that it exists mostly in the FR conformer. These

findings, along with increased EndoGluC protease susceptibility of PMA-treated enzymes, agree with the **model** that the FO and FR conformations are in equilibrium. PMA treatment, because of steric limitations of the phenylmercuric adduct in the FO form, forces the equilibrium to the FR conformer, where AADP+ binding can cause fluorescence quenching and conformational restriction favors proteolytic susceptibility.

L37 ANSWER 47 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Tansley review no 94 - Thioredoxins: Structure and function in plant cells
SO NEW PHYTOLOGIST, (AUG 1997) Vol. 136, No. 4, pp. 543-570.
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211.
ISSN: 0028-646X.
AU Jacquot J P (Reprint); Lancelin J M; Meyer Y
AB Thioredoxins are ubiquitous small-molecular-weight proteins (typically 100-120 amino-acid residues) containing an extremely reactive disulphide bridge with a highly conserved sequence -Cys-Gly(Ala/Pro)-Pro-Cys-. In bacteria and animal cells, thioredoxins participate in multiple reactions which require reduction of disulphide bonds on selected target proteins/enzymes. There is now ample biochemical evidence that thioredoxins exert very specific functions in plants, the best documented being the redox regulation of chloroplast enzymes. Another area in which thioredoxins are believed to play a prominent role is in reserve protein mobilization during the process of germination. It has been discovered that thioredoxins constitute a large multigene family in plants with different subcellular localizations, a unique feature in living cells so far. Evolutionary studies based on these molecules will be discussed, as well as the available biochemical and genetic evidence related to their functions in plant cells. Eukaryotic photosynthetic plant cells are also unique in that they possess two different reducing systems, one extrachloroplastic dependent on NADPH as an electron donor, and the other one chloroplastic, dependent on photoreduced ferredoxin. This review will examine in detail the latest progresses in the area of thioredoxin structural biology in plants, this protein being an excellent **model** for this purpose. The structural features of the reducing enzymes ferredoxin **thioredoxin reductase** and NADPH **thioredoxin reductase** will also be described. The properties of the target enzymes known so far in plants will be detailed with special emphasis on the structural features which make them redox regulatory. Based on sequence analysis, evidence will be presented that redox regulation of enzymes of the biosynthetic pathways first appeared in cyanobacteria possibly as a way to cope with the oxidants produced by oxygenic photosynthesis. It became more elaborate in the chloroplasts of higher plants where a co-ordinated functioning of the chloroplastic and extra chloroplastic metabolisms is required.

L37 ANSWER 48 OF 94 MEDLINE on STN DUPLICATE 21
TI The 58 kDa mouse selenoprotein is a BCNU-sensitive **thioredoxin reductase**.
SO FEBS letters, (1997 Jul 28) 412 (2) 318-20.
Journal code: 0155157. ISSN: 0014-5793.
AU Gromer S; Schirmer R H; Becker K
AB The flavoprotein **thioredoxin reductase** [EC 1.6.4.5] (NADPH + H+ + thioredoxin-S2 --> NADP+ + thioredoxin-(SH)2) was isolated from mouse Ehrlich ascites tumour (EAT) cells. Like the counterpart from human placenta but unlike the known **thioredoxin reductases** from non-vertebrate organisms, the mouse enzyme was found to contain 1 equivalent of selenium per subunit of 58 kDa. The K(M) values were 4.5 microM for NADPH, 480 microM for DTNB and 36 microM for Escherichia **coli** thioredoxin, the turnover number with DTNB being approximately 40 s(-1). As mouse is a standard animal **model** in cancer and malaria research, **thioredoxin reductase** and glutathione reductase [EC 1.6.4.2] from EAT cells were compared with each other. While both enzymes in their 2-electron reduced form are targets of the cytostatic drug carmustine (BCNU), no immunologic cross-reactivity between the two mouse disulfide reductases was observed.

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TI The comparative metabolism of the mollicutes (Mycoplasmas): The utility for taxonomic classification and the relationship of putative gene annotation and phylogeny to enzymatic function in the smallest free-living cells.

SO Critical Reviews in Microbiology, (1997) 23/4 (269-354).
Refs: 333
ISSN: 1040-841X CODEN: CRVMAC

AU Pollack J.D.; Williams M.V.; McElhaney R.N.

AB Mollicutes or mycoplasmas are a class of wall-less bacteria descended from low G + C% Gram-positive bacteria. Some are exceeding small, about 0.2 .mu.m in diameter, and are examples of the smallest free-living cells known. Their genomes are equally small; the smallest in Mycoplasma genitalium is sequenced and is 0.58 mb with 475 ORFs, compared with 4.639 mb and 4288 ORFs for Escherichia **coli**. Because of their size and apparently limited metabolic potential, Mollicutes are **models** for describing the minimal metabolism necessary to sustain independent life. Mollicutes have no cytochromes or the TCA cycle except for malate dehydrogenase activity. Some uniquely require cholesterol for growth, some require urea and some are anaerobic. They fix CO2 in anaplerotic or replenishing reactions. Some require pyrophosphate not ATP as an energy source for reactions, including the rate-limiting step of glycolysis: 6-phosphofructokinase. They scavenge for nucleic acid precursors and apparently do not synthesize pyrimidines or purines de novo. Some genera uniquely lack dUTPase activity and some species also lack uracil-DNA glycosylase. The absence of the latter two reactions that limit the incorporation of uracil or remove it from DNA may be related to the marked mutability of the Mollicutes and their tachytelic or rapid evolution. Approximately 150 cytoplasmic activities have been identified in these organisms, 225 to 250 are presumed to be present. About 100 of the core reactions are graphically linked in a metabolic map, including glycolysis, pentose phosphate pathway, arginine dihydrolase pathway, transamination, and purine, pyrimidine, and lipid metabolism. Reaction sequences or loci of particular importance are also described: phosphofructokinases, NADH oxidase, thioredoxin complex, deoxyribose-5-phosphate aldolase, and lactate, malate, and glutamate dehydrogenases. Enzymatic activities of the Mollicutes are grouped according to metabolic similarities that are taxonomically discriminating. The arrangements attempt to follow phylogenetic relationship. The relationship of putative gene assignments and enzymatic function in My. genitalium, My. pneumoniae, and My. capricolum subsp. capricolum are specifically analyzed. The data are arranged in four tables. One associates gene annotations with congruent reports of the enzymatic activity in these same Mollicutes, and hence confirms the annotations. Another associates putative annotations with reports of the enzyme activity but from different Mollicutes. A third identifies the discrepancies represented by those enzymatic activities found in Mollicutes with sequenced genomes but without any similarly annotated ORF. This suggests that the gene sequence is significantly different from those already deposited in the databanks and putatively annotated with the same function. Another comparison lists those enzymatic activities that are both undetected in Mollicutes and not associated with any ORF. Evidence is presented supporting the theory that there are relatively small gene sequences that code for functional centers of multiple enzymatic activity. This property is seemingly advantageous for an organism with a small genome and perhaps under some coding restraint. The data suggest that a concept of 'remnant' or 'useless genes' or 'useless enzymes' should be considered when examining the relationship of gene annotation and enzymatic function. It also suggests that genes in addition to representing what cells are doing or what they may do, may also identify what they once might have done and may never do again.

L37 ANSWER 50 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 22

TI A STABLE MIXED DISULFIDE BETWEEN **THIOREDOXIN REDUCTASE** AND ITS SUBSTRATE, THIOREDOXIN - PREPARATION AND CHARACTERIZATION

SO BIOCHEMISTRY, (16 APR 1996) Vol. 35, No. 15, pp. 4812-4819.
ISSN: 0006-2960.

AU WANG P F; VEINE D M; AHN S H; WILLIAMS C H (Reprint)

AB The flavoenzyme **thioredoxin reductase** (TrR) catalyzes the reduction of the small redox protein thioredoxin (Tr) by NADPH. It has been proposed that a large conformational change is required in catalysis by TrR in order to visualize a complete pathway for reduction

of equivalents. The proposal is based on the comparison of the **crystal** structures of TrR and glutathione reductase, the latter being a well-understood member of this enzyme family [Waksman, G., et al, (1994) J. Mol. Biol. 236, 800-816]. Bound NADPH is perfectly positioned for electron transfer to the FAD in glutathione reductase, but in TrR, these two components are 17 Angstrom apart. In order to provide evidence for the proposed conformational change, a complex between TrR and its substrate Tr involving a mixed disulfide between TrR and Tr was prepared. The redox active disulfide of TrR is composed of Cys(135) and Cys(138), and the redox active disulfide of Tr is made up of Cys(32) and Cys(35). The complex C135S-C32S is prepared from forms of TrR and Tr altered by site-directed mutagenesis where Cys(138) and Cys(35) remaining in TrR and Tr, respectively, The purified C135S-C32S presents a band on a nonreducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis corresponding to a molecular weight sum of one subunit of TrR and one of Tr. Several observations indicate that C135S-C32S can adopt only one conformation It was reported previously that TrR C135S can form a charge transfer complex in the presence of ammonium cation in which the donor is the remaining thiolate of Cys(138) [Prongay, A. J., et al., (1989) J. Biol. Chem. 264, 2656-2664], while titration of C135S-C32S with NH4Cl does not induce charge transfer, presumably because Cys(138) is participating in the mixed disulfide. Reduction of C135S-C32S with dithiothreitol (DTT) results in a decrease of epsilon(454) to a value similar to that of TrR C135S, and subsequent NH4Cl titration leads to charge transfer complex formation in the nascent TrR C135S. Reductive titrations show that approximately 1 equiv of sodium dithionite or NADPH is required to fully reduce C135S-C32S, and treatment with NH4Cl and DTT demonstrates that the mixed disulfide remains intact. These results indicate that C135S-C32S is a stable mixed disulfide between Cys(138) of TrR C135S and Cys(35) of Tr C32S that locks the structure in a conformation where FAD can be reduced by NADPH, but electrons cannot flow from FADH(2) to the mixed disulfide bond.

L37 ANSWER 51 OF 94 MEDLINE on STN DUPLICATE 23

TI Replacement of Trp28 in Escherichia **coli** thioredoxin by site-directed mutagenesis affects thermodynamic stability but not function.

SO Journal of biological chemistry, (1996 Feb 9) 271 (6) 3091-6. Journal code: 2985121R. ISSN: 0021-9258.

AU Slaby I; Cerna V; Jeng M F; Dyson H J; Holmgren A

AB Escherichia **coli** thioredoxin contains two tryptophan residues (Trp28 and Trp31) situated close to the active site disulfide/dithiol. In order to probe the structural and functional roles of tryptophan in the mechanism of E. **coli** thioredoxin (Trx), we have replaced Trp28 with alanine using site-directed mutagenesis and expressed the mutant protein W28A in E. **coli**. Changes in the behavior of the mutant protein compared with the wild-type protein have been monitored by a number of physical and spectroscopic techniques and enzyme assays. As expected, removal of a tryptophan residue causes profound changes in the fluorescence spectrum of thioredoxin, particularly for the reduced protein (Trx-(SH)2), and to a lesser extent for the oxidized protein (Trx-S2). These results show that the major contribution to the strongly quenched fluorescence of Trx-S2 in both wild-type and mutant proteins is from Trp31, whereas the higher fluorescence quantum yield of Trx-(SH)2 in the wild-type protein is dominated by the emission from Trp28. The fluorescence, CD, and 1H **NMR** spectra are all indicative that the mutant protein is fully folded at pH 7 and room temperature, and, despite the significance of the change, from a tryptophan in close proximity to the active site to an alanine, the functions of the protein appear to be largely intact. W28A Trx-S2 is a good substrate for **thioredoxin reductase**, and W28A Trx-(SH)2 is as efficient as wild-type protein in reduction of insulin disulfides. DNA polymerase activity exhibited by the complex of phage T7 gene 5 protein and Trx-(SH)2 is affected only marginally by the W28A substitution, consistent with the buried position of Trp28 in the protein. However, the thermodynamic stability of the molecule appears to have been greatly reduced by the mutation: guanidine hydrochloride unfolds the protein at a significantly lower concentration for the mutant than for wild type, and the thermal stability is reduced by about 10 degrees C in each case. The stability of each form of the protein appears to be reduced by the same amount, an indication that the effect of the mutation is identical in both forms of the protein. Thus,

despite its close proximity to the active site, the Trp28 residue of thioredoxin is not apparently essential to the electron transfer mechanism, but rather contributes to the stability of the protein fold in the active site region.

L37 ANSWER 52 OF 94 MEDLINE on STN DUPLICATE 24
TI **Crystal** structure of Arabidopsis **thaliana** NADPH dependent **thioredoxin reductase** at 2.5 A resolution.
SO Journal of molecular biology, (1996 Dec 20) 264 (5) 1044-57.
Journal code: 2985088R. ISSN: 0022-2836.
AU Dai S; Saarinen M; Ramaswamy S; Meyer Y; Jacquot J P; Eklund H
AB Thioredoxin exists in all organisms and is responsible for the hydrogen transfer to important enzymes for ribonucleotide reduction and the reduction of methionine sulphoxide and sulphate. Thioredoxins have also been shown to regulate enzyme activity in plants and are also involved in the regulation of transcription factors and several other regulatory activities. Thioredoxin is reduced by the flavoenzyme **thioredoxin reductase** using NADPH. We have now determined the first structure of a eukaryotic **thioredoxin reductase**, from the plant Arabidopsis **thaliana**, at 2.5 A resolution. The dimeric A. **thaliana thioredoxin reductase** is structurally similar to that of the Escherichia **coli** enzyme, and most differences occur in the loops. Because the plant and E. **coli** enzymes have the same architecture, with the same dimeric structure and the same position of the redox active disulphide bond, a similar mechanism that involves very large domain rotations is likely for the two enzymes. The subunit is divided into two domains, one that binds FAD and one that binds NADPH. The relative positions of the domains in A. **thaliana thioredoxin reductase** differ from those of the E. **coli** reductase. When the FAD domains are superimposed, the NADPH domain of A. **thaliana thioredoxin reductase** must be rotated by 8 degrees to superimpose on the corresponding domain of the E. **coli** enzyme. The domain rotation we now observe is much smaller than necessary for the thioredoxin reduction cycle.

L37 ANSWER 53 OF 94 MEDLINE on STN
TI Conservative substitutions in the hydrophobic core of Rhodobacter sphaeroides thioredoxin produce distinct functional effects.
SO Protein science : a publication of the Protein Society, (1995 Dec) 4 (12) 2510-6.
Journal code: 9211750. ISSN: 0961-8368.
AU Assemat K; Alzari P M; Clement-Metral J
AB The internal residue Phe 25 in Rhodobacter sphaeroides thioredoxin was changed to five amino acids (Ala, Val, Leu, Ile, Tyr) by site-directed mutagenesis, and the mutant proteins were characterized in vitro and in vivo using the mutant trxA genes in an Escherichia **coli** TrxA- background. The substitution F25A severely impaired the functional properties of the enzyme. Strains expressing all other mutations can grow on methionine sulfoxide with growth efficiencies of 45-60% that of the wild type at 37 degrees, and essentially identical at 42 degrees. At both temperatures, however, strains harboring the substitutions F25V and F25Y had lower growth rates and formed smaller colonies. In another in vivo assay, only the wild type and the F25I substitution allowed growth of phage T3/7 at 37 degrees, demonstrating that subtle modifications of the protein interior at position 25 Ile/Leu or Phe/Tyr) can produce significant biological effects. All F25 mutants were good substrates for E. **coli thioredoxin reductase**. Although turnover rates and apparent Km values were significantly lower for all mutants compared to the wild type, catalytic efficiency of **thioredoxin reductase** was similar for all substrates. Determination of the free energy of unfolding showed that the aliphatic substitutions (Val, Leu, Ile) significantly destabilized the protein, whereas the F25Y substitution did not affect protein stability. Thus, thermodynamic stability of R. sphaeroides thioredoxin variants is not correlated with the distinct functional effects observed both in vivo and in vitro.

L37 ANSWER 54 OF 94 MEDLINE on STN DUPLICATE 25
TI Mechanism and structure of **thioredoxin reductase** from Escherichia **coli**.
SO FASEB journal : official publication of the Federation of American

Societies for Experimental Biology, (1995 Oct) 9 (13) 1267-76. Ref: 47
Journal code: 8804484. ISSN: 0892-6638.
AU Williams C H Jr
AB The flavoprotein **thioredoxin reductase** catalyzes the reduction of the small redox protein thioredoxin by NADPH. **Thioredoxin reductase** contains a redox active disulfide and is a member of the pyridine nucleotide-disulfide oxidoreductase family of flavoenzymes that includes lipoamide dehydrogenase, glutathione reductase, trypanothione reductase, mercuric reductase, and NADH peroxidase. The structure of **thioredoxin reductase** has recently been determined from **X-ray** crystallographic data. In this paper, we attempt to correlate the structure with a considerable body of mechanistic data and to arrive at a mechanism consistent with both. The path of reducing equivalents in catalysis by glutathione reductase and lipoamide dehydrogenase is clear. To envisage the path of reducing equivalents in catalysis by **thioredoxin reductase**, a conformational change is required in which the NADPH domain rotates relative to the FAD domain. The rotation moves the nascent dithiol from its observed position adjacent to the re surface of the flavin ring system toward the protein surface for dithiol-disulfide interchange with the protein substrate thioredoxin and moves the nicotinamide ring of NADPH adjacent to the flavin ring for efficient hydride transfer. Reverse rotation allows reduction of the redox active disulfide by the reduced flavin. This requires that the enzyme pass through a ternary complex; the kinetic evidence for such a complex is discussed.

L37 ANSWER 55 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI THIOREDOXIN AND **THIOREDOXIN REDUCTASE**
SO METHODS IN ENZYMOLOGY, (1995) Vol. 252, pp. 199-208.
ISSN: 0076-6879.
AU HOLMGREN A (Reprint); BJORNSTEDT M

L37 ANSWER 56 OF 94 CAPLUS COPYRIGHT 2004 ACS on STN
TI Synthetic competition between cytoplasmic folding and translocation of a soluble membrane protein domain
SO Research in Microbiology (1995), 146(2), 121-8
CODEN: RMCREW; ISSN: 0923-2508
AU Uhland, K.; Zander, T.; Ehrmann, M.
AB In wild-type strains of Escherichia **coli**, alk. phosphatase (AP), either when present as a sol. protein or when fused to a membrane protein, is only active after translocation to the periplasm. In **thioredoxin reductase** (trxB) mutants, however, cytoplasmically localized AP can form disulfide bonds and achieve an active conformation. Once it has folded in the cytoplasm, it can no longer be translocated. On the other hand, when AP is fused to periplasmic domains of a membrane protein, translocation can be more rapid than folding. Thus, expressing hybrids of AP and integral membrane proteins in a trxB mutant generates competition between folding of AP in the cytoplasm and its translocation to the periplasm. The cellular localization of AP can be monitored in phosphoserine phosphatase (serB) mutants causing auxotrophy for L-serine. Cytoplasmically but not periplasmically localized AP can compensate for the lack of SerB, leading to growth on indicator plates. As expected, when AP was fused to cytoplasmic domains of membrane proteins, serB-mediated auxotrophy was abolished. Surprisingly, AP fusions to periplasmic domains exhibited a non-uniform response pattern. Fusions that translocate AP rapidly did not complement the SerB defect, while those that export AP only slowly could do so. The usefulness of these strains for studying a variety of aspects related to membrane protein biogenesis is discussed.

L37 ANSWER 57 OF 94 MEDLINE on STN
TI Coenzyme B12-dependent ribonucleotide reductase: evidence for the participation of five cysteine residues in ribonucleotide reduction.
SO Biochemistry, (1994 Oct 25) 33 (42) 12676-85.
Journal code: 0370623. ISSN: 0006-2960.
AU Booker S; Licht S; Broderick J; Stubbe J
AB Ribonucleoside triphosphate reductase (RTPR) from Lactobacillus leichmannii catalyzes the conversion of ribonucleotides to 2'-deoxyribonucleotides and requires adenosylcobalamin (AdoCbl) as a

cofactor. Recent cloning, sequencing, and expression of this protein [Booker, S., & Stubbe, J. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 8352-8356] have now allowed its characterization by site-directed mutagenesis. The present study focuses on the role of five cysteines postulated to be required for catalysis. The choice of which of the ten cysteines of RTPR were to be mutated was based on extensive studies on the Escherichia **coli** ribonucleoside diphosphate reductase. Despite the differences between these two reductases in primary sequence, quaternary structure, and cofactor requirements, their mechanisms are strikingly similar. The mutagenesis studies reported herein further suggest that the complex role of the five cysteines is also very similar. A variety of single and double mutants of RTPR were prepared (C731S, C736S, C731 and 736S, C119S, C419S, C408S, and C305S), and their interaction with the normal substrate (CTP) was characterized under several sets of conditions. Mutants C731S, C736S, and C731 and 736S all catalyzed the formation of dCTP at rates similar to those of the wild-type (wt) enzyme in the presence of the artificial reductant DTT. In the presence of the in vivo reducing system (thioredoxin, **thioredoxin reductase**, and NADPH), however, each of these mutants catalyzed the formation of only 0.6-0.8 dCTPs per mole of enzyme. The inability of these mutants to catalyze multiple turnovers with respect to the in vivo reducing system suggests that their function might be to transfer reducing equivalents from thioredoxin into the active site disulfide of the reductase. Mutants C119S and C419S were targeted as being the active site cysteines, the ones which directly reduce the ribonucleotide substrate. As expected, neither of these mutants catalyzed the formation of dCTP. However, they did catalyze a time-dependent formation of cytosine, destruction of the cofactor, and the appearance of a chromophore associated with the protein--all phenotypes previously observed for the corresponding active site cysteines of the E. **coli** reductase. Mutant C408S was unable to catalyze dNTP production or cytosine release. Moreover, it was ineffective in catalyzing two additional reactions which are unique to this enzyme: the exchange of tritium from the 5' hydrogens of AdoCbl with H2O and the destruction of AdoCbl under anaerobic conditions to give 5'-deoxyadenosine and cob(II)alamin. These results are consistent with the role of this cysteine as the protein radical responsible for initiating catalysis.

L37 ANSWER 58 OF 94 MEDLINE on STN DUPLICATE 26
TI Potential active-site base of **thioredoxin reductase** from Escherichia **coli**: examination of histidine245 and aspartate139 by site-directed mutagenesis.
SO Biochemistry, (1994 Mar 22) 33 (11) 3148-54.
Journal code: 0370623. ISSN: 0006-2960.
AU Mulrooney S B; Williams C H Jr
AB It has been proposed that an acid-base catalyst facilitates the reduction of thioredoxin by **thioredoxin reductase** from Escherichia **coli** [O'Donnell, M. E., & Williams, C. H. Jr. (1983) J. Biol. Chem. 252, 13795-13805]. The **X-ray crystal** structure reveals two groups which could potentially fulfill this role: His245 and Asp139. Using site-directed mutagenesis, His245 was changed to asparagine (H245N) and alanine (H245A) and Asp139 was changed to glutamate (D139E), asparagine (D139N), and leucine (D139L). Steady-state kinetic analysis of the His245 mutants gave turnover numbers and Km values similar to those of wild-type **thioredoxin reductase**. All three Asp139 mutants were altered in their overall kinetic properties: D139E had 38% of wild-type activity, D139N had 1.5%, and D139L had no measureable activity. Rate constants for the NADPH to 3-acetylpyridine adenine dinucleotide phosphate transhydrogenase activity were similar for all of the Asp139 and His245 mutants and wild-type **thioredoxin reductase**. Stopped-flow kinetic measurements of the reductase half-reaction of H245A and H245N gave rate constants that were up to 2-fold faster than those found for wild-type **thioredoxin reductase**, while all of the Asp139 mutants had rate constants comparable to those of wild-type. To further examine the causes of the low overall activity of D139N, the oxidative half-reaction was measured. The reoxidation of reduced D139N mixed with oxidized thioredoxin occurred at a very slow rate constant of 0.23 s-1-about 1% that of wild-type enzyme. We suggest that Asp139 is the active-site acid catalyst which functions to protonate the thiolate anion of reduced thioredoxin.(ABSTRACT TRUNCATED AT 250 WORDS)

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TI REDOX-ACTIVE BIS-CYSTEINYL PEPTIDES .2. COMPARATIVE-STUDY ON THE SEQUENCE-DEPENDENT TENDENCY FOR DISULFIDE LOOP FORMATION

SO BIOPOLYMERS, (NOV 1994) Vol. 34, No. 11, pp. 1563-1572.
ISSN: 0006-3525.

AU SIEDLER F; QUARZAGO D; RUDOLPHBOHNER S; MORODER L (Reprint)

AB Bis(cysteinyl) octapeptides related to the active sites of the oxidoreductases protein disulfide isomerase (PDI), **thioredoxin reductase** (trr), glutaredoxin (grx), and thioredoxin (trx) were analyzed for their propensity to form the intramolecular 14-membered disulfide ring in oxidation experiments. The rank order of percentage of cyclic monomer formed in aqueous buffer (pH 7.0) at 10(-3) M concentration was found to be very similar, but opposite to that of the K-ox and, correspondingly, of the redox potentials of the native enzymes. Attempts to induce intrinsic conformational preferences of the peptides by addition of trifluoroethanol led to enhancements of beta-turn structures as reflected by the CD and Fourier transform ir spectre. The induced secondary structure, instead of aligning the tendencies of the excised fragments for loop formation with those of the intact proteins, was found to suppress the differences by significantly increasing the preference for cyclic monomers (approximate to 90%). Similarly, operating under denaturing conditions, i.e., in 6M guanidinium hydrochloride, only for the trx peptide was the statistical product distribution obtained. For the remaining peptides, again a strong increase of cyclic monomer contents was observed that could not be correlated with dissolution of beta-sheet type aggregates. The CD spectra are more consistent with the presence of ordered structure to some extent, possibly resulting from an hydrophobic collapse of the sparingly soluble peptides. The results of the oxidation experiments further support previous findings from thiol disulfide interchange equilibria, which clearly revealed a decisive role of the characteristic thioredoxin structural motif in dictating the redox properties of the enzymes. Point mutations in the active sites of the oxidoreductases allowed us to affect their redox potentials strongly, but apparently only in the constraint form of the three-dimensional structure as similar exchanges in the excised fragments did not produce the expected effect. This observation contrasts with numerous reports that the conformation of short disulfide loops is mainly dictated by the amino acid sequence. (C) 1994 John Wiley and Sons, Inc.

L37 ANSWER 60 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI REDOX-ACTIVE BIS-CYSTEINYL PEPTIDES .1. SYNTHESIS OF CYCLIC CYSTINYL PEPTIDES BY CONVENTIONAL METHODS IN SOLUTION AND ON SOLID SUPPORTS

SO BIOPOLYMERS, (NOV 1994) Vol. 34, No. 11, pp. 1553-1562.
ISSN: 0006-3525.

AU MUSIOL H J; SIEDLER F; QUARZAGO D; MORODER L (Reprint)

AB Cyclic mono-cystinyl active-site fragments of thioredoxin and **thioredoxin reductase** were synthesized as N-acetyl and C-amide octapeptides by conventional methods of peptide synthesis in solution and on solid supports. Using a side-chain protection based on acidlabile tert-butanol-derived groups and on the S-tert-butylthio unsymmetric disulfide for the thiol functions, in combination with N-alpha-Z- or N-alpha-Nps derivatives in the chain elongation steps, the synthesis in solution was carried out in straightforward manner yielding the fully protected octapeptides as well characterized compounds. Upon deprotection with trifluoroacetic acid and reduction of the unsymmetrical disulfides with tri-butylphosphine, the resulting bis-cysteinyl-octapeptides were oxidized in dimethylformamide with azodicarboxylic acid di-tert-butyl ester to produce the desired cyclic compounds in good overall yields. For the synthesis on solid supports a similar acid-labile side-chain protection was applied in combination with the N-alpha 9-flourenylmethyoxycarbonyl derivatives in the chain elongation steps. Thereby acylations were performed with the related amino acid N-carboxyanhydrides (UNCAs) or by the O-(1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium-tetrafluoroborate/1-hydroxybenzotriazole (TBTU/HOBt) procedure. The solid phase synthesis of the two octapeptides led to unexpected difficulties in terms of recovery of peptidic material from the resins in the final acidolytic cleavage step as well as of racemization at the level of the cysteine residues by the TBTU/HOBt coupling method.

Racemization was efficiently suppressed by employing the related pentafluorophenyl ester and this method led to crude octapeptide products of a degree of purity comparable to those obtained by the synthesis in solution. However, the recovery of the peptides from the resin, i.e., irreversible reattachment of cleaved peptidic material via alkylation of various sidechain functions, could not be avoided even using the most efficient scavengers or their cocktails. (C) 1994 John Wiley and Sons, Inc.

L37 ANSWER 61 OF 94 MEDLINE on STN DUPLICATE 27
TI **Crystal** structure of Escherichia **coli** **thioredoxin reductase** refined at 2 A resolution. Implications for a large conformational change during catalysis.
SO Journal of molecular biology, (1994 Feb 25) 236 (3) 800-16. Journal code: 2985088R. ISSN: 0022-2836.
AU Waksman G; Krishna T S; Williams C H Jr; Kuriyan J
AB The **crystal** structures of three forms of Escherichia **coli** **thioredoxin reductase** have been refined: the oxidized form of the wild-type enzyme at 2.1 A resolution, a variant containing a cysteine to serine mutation at the active site (Cys138Ser) at 2.0 A resolution, and a complex of this variant with nicotinamide adenine dinucleotide phosphate (NADP+) at 2.3 A resolution. The enzyme mechanism involves the transfer of reducing equivalents from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to a disulfide bond in the enzyme, via a flavin adenine dinucleotide (FAD). **Thioredoxin reductase** contains FAD and NADPH binding domains that are structurally similar to the corresponding domains of the related enzyme glutathione reductase. The relative orientation of these domains is, however, very different in the two enzymes: when the FAD domains of thioredoxin and glutathione reductases are superimposed, the NADPH domain of one is rotated by 66 degrees with respect to the other. The observed binding mode of NADP+ in **thioredoxin reductase** is non-productive in that the nicotinamide ring is more than 17 A from the flavin ring system. While in glutathione reductase the redox active disulfide is located in the FAD domain, in **thioredoxin reductase** it is in the NADPH domain and is part of a four-residue sequence (Cys-Ala-Thr-Cys) that is close in structure to the corresponding region of thioredoxin (Cys-Gly-Pro-Cys), with a root-mean-square deviation of 0.22 A for atoms in the disulfide bonded ring. There are no significant conformational differences between the structure of the wild-type enzyme and that of the Cys138Ser mutant, except that a disulfide bond is not present in the latter. The disulfide bond is positioned productively in this conformation of the enzyme, i.e. it stacks against the flavin ring system in a position that would facilitate its reduction by the flavin. However, the cysteine residues are relatively inaccessible for interaction with the substrate, thioredoxin. These results suggest that **thioredoxin reductase** must undergo conformational changes during enzyme catalysis. All three structures reported here are for the same conformation of the enzyme and no direct evidence is available as yet for such conformational changes. The simplest possibility is that the NADPH domain rotates between the conformation observed here and an orientation similar to that seen in glutathione reductase. This would alternately place the nicotinamide ring and the disulfide bond near the flavin ring, and expose the cysteine residues for reaction with thioredoxin in the hypothetical conformation. (ABSTRACT TRUNCATED AT 400 WORDS)

L37 ANSWER 62 OF 94 MEDLINE on STN
TI Building bridges: disulphide bond formation in the cell.
SO Molecular microbiology, (1994 Oct) 14 (2) 199-205. Ref: 46 Journal code: 8712028. ISSN: 0950-382X.
AU Bardwell J C
AB Disulphides are often vital for the folding and stability of proteins. Dedicated enzymatic systems have been discovered that catalyse the formation of disulphides in the periplasm of prokaryotes. These discoveries provide compelling evidence for the actual catalysis of protein folding in vivo. Disulphide bond formation in Escherichia **coli** is catalysed by at least three 'Dsb' proteins; DsbA, -B and -C. The DsbA protein has an extremely reactive, oxidizing disulphide which it simply donates directly to other proteins. DsbB is required for the reoxidation of DsbA. DsbC is active in disulphide rearrangements and

appears to work synergistically with DsbA. The relative rarity of disulphides in cytoplasmic proteins appears to be dependent upon a disulphide-destruction machine. One pivotal cog in this machine is **thioredoxin reductase**.

L37 ANSWER 63 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI ALTERNATIVE **MODEL** FOR MECHANISM-BASED INHIBITION OF ESCHERICHIA-**COLI** RIBONUCLEOTIDE REDUCTASE BY 2'-AZIDO-2'-DEOXYURIDINE 5'-DIPHOSPHATE
SO BIOCHEMISTRY, (30 NOV 1993) Vol. 32, No. 47, pp. 12749-12760. ISSN: 0006-2960.
AU SALOWE S; BOLLINGER J M; ATOR M; STUBBE J (Reprint); MCCRACKEN J; PEISACH J; SAMANO M C; ROBINS M J
AB Ribonucleotide reductase (RDPR) from Escherichia **coli** is composed of two subunits, R1 and R2, and catalyzes the conversion of nucleotides to deoxynucleotides. The mechanism of inactivation of RDPR by 2'-azido-2'-deoxynucleoside 5'-diphosphate (N3UDP) has been examined using a variety of isotopically labeled derivatives: (1'-, 2'-, 3'-, or 4'-[H-2])-N3UDPs and 2'-[N-15(3), C-13]-N3UDP. Electron paramagnetic resonance (EPR) and electron spin echo envelope modulation (ESEEM) spectroscopy studies using these compounds indicate that the 2' carbon-nitrogen bond to the azide moiety is cleaved prior to or upon formation of the nitrogen-centered radical derived from the azide moiety of N3UDP. EPR studies reveal no hyperfine interactions of the nitrogen-centered radical with the 1', 2', 3', or 4' hydrogens of N3UDP. ESEEM studies however, reveal that the 1' and 4' deuterons are 3.3 +/- 0.2 and 2.6 +/- 0.3 angstrom, respectively, from the nitrogen-centered radical. Further support for carbon-nitrogen bond cleavage is derived from studies of the interaction of oxidized R1, C225SR1, and C462SR1 with R2 and N3UDP. In all three cases, in contrast to the results with the wild type R1, azide is detected. Nitrogen-centered radical is not observed with either oxidized R1 or C225SR1 but is observed with C462SR1. These results suggest that C225 is required for the conversion of azide into N2 and a nitrogen-centered radical. The dynamics of the inactivation of RDPR by N3UDP have also been examined. Use of [3'-H-2]N3UDP reveals an isotope effect of approximately 2 on the loss of the tyrosyl radical and the rate of inactivation of RDPR. In both cases the kinetics are complex, suggesting multiple modes of inactivation. In addition, several modes of inactivation are required to explain the observation that loss of the tyrosyl radical is slower than the rate of inactivation. Studies using [5'-H-3]N3UDP reveal that the rapid inactivation is the result of the formation of a tight noncovalent complex between modified nucleotide, nitrogen-centered radical and RDPR. Destruction of the nitrogen-centered radical is a slow process which appears to be accompanied by decomposition of the modified nucleotide into PPi, uracil, and 2-methylene-3(2H)-furanone. The latter covalently modifies R1 and ultimately leads to loss of approximately 50% of the activity of R1.

L37 ANSWER 64 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI REDOX POTENTIALS OF ACTIVE-SITE BIS(CYSTEINYL) FRAGMENTS OF THIOL-PROTEIN OXIDOREDUCTASES
SO BIOCHEMISTRY, (27 JUL 1993) Vol. 32, No. 29, pp. 7488-7495. ISSN: 0006-2960.
AU SIEDLER F; RUDOLPHBOHNER S; DOI M; MUSIOL H J; MORODER L (Reprint)
AB The active sites of thiol-protein oxidoreductases consist of the characteristic Cys-X-X-CYS motif, and the redox potentials of these enzymes reflect the propensity of the bis(cysteinyl) sequence portion for disulfide loop formation. Thereby, as is known from comparing the three-dimensional (3D) structures of thioredoxin and glutaredoxin in the reduced and oxidized state, reduction of the disulfide bond is accompanied by minimal perturbation of the backbone folding of the active sites. In order to estimate the sequence-dependent intrinsic free energy of formation of the active-site disulfide loops in oxidoreductases, synthetic fragments corresponding to the sequences 31-38, 10-17, 134-141, and 34-41 of thioredoxin, glutaredoxin, **thioredoxin reductase**, and protein disulfide isomerase (PDI), respectively, were analyzed for their tendency to form 14-membered rings. For this purpose thiol/disulfide exchange experiments, with glutathione as reference redox pair, were performed on the bis(cysteinyl) octapeptides. As the free energy of ring

closure of linear peptides consists mainly of the free energy of formation of the disulfide loop with a defined geometry from a statistical ensemble of conformations of the bis(cysteinyl) peptides, the observed differences in the equilibrium constants, although relatively small (within a factor 10), suggest that sequence-dependent information for loop formation is retained in the excised active-site fragments. These inherent redox potentials are, however, significantly affected and/or amplified in the native proteins by the conformational restraints imposed by the ''structural domains'' on the ''functional domains''.

L37 ANSWER 65 OF 94 MEDLINE on STN
TI Reduction of mutant phage T4 glutaredoxins by Escherichia **coli thioredoxin reductase**.
SO Journal of biological chemistry, (1993 Feb 25) 268 (6) 3845-9.
Journal code: 2985121R. ISSN: 0021-9258.
AU Nikkola M; Gleason F K; Eklund H
AB Fifteen mutant T4 glutaredoxins (previously T4 thioredoxin) have been assayed for activity with Escherichia **coli thioredoxin reductase**. The mutations include substitutions in the region of the active site, in the 2 cysteines, and in the 2 residues between the cysteines forming the active-site disulfide bridge. Mutant thioredoxins where substitutions have been made in charged residues around the active site show the biggest differences in activity. The positive residues Lys-13 and Lys-21 were found to be important for efficient binding to **thioredoxin reductase**. Substitution of the aspartic acid at position 80 with a serine produced a glutaredoxin with superior activity. This mutant glutaredoxin has earlier been shown to be more efficient than the wild type in thiol transferase activity (Nikkola, M., Gleason, F. K., Saarinen, M., Joelson, T., Bjornberg, O., and Eklund, H. (1991) J. Biol. Chem. 266, 16105-16112). Even the glutaredoxin P66A, where the active-site cis-proline has been substituted, could be efficiently reduced by **thioredoxin reductase**. Glutaredoxins lacking one or both cysteines were not active.

L37 ANSWER 66 OF 94 EMBASE
on STN
TI Selenium metabolism in micro-organisms.
SO Advances in Microbial Physiology, (1993) 35/- (71-109).
ISSN: 0065-2911 CODEN: AMIPB2
AU Heider J.; Bock A.

L37 ANSWER 67 OF 94 BIOSIS on STN
TI Studies on the role of disulfide in CDP-6-deoxy-DELTA-34-glucoseen reductase (E3).
SO Korean Biochemical Journal, (1993) Vol. 26, No. 1, pp. 34-40.
CODEN: KBCJAK. ISSN: 0368-4881.
AU Han, Oksoo
AB The NADH oxidase activity of CDP-6-Deoxy-DELTA-3,4-glucoseen reductase (E3) was decreased by thiol modifying agents such as iodoacetic acid or DTNB. The enzyme was reduced NADH and the number of free thiols was gradually increased by the reduction. The titration of free thiols in E3 at various redox-stages by DTNB revealed that the enzyme contained at least one reducible disulfide near the active-site. This result, together with studies on the inactivation of E3 by excess NADH, indicates the redox-active disulfide may be involved in the catalytic cycle of E3 although the fully reduced form of the enzyme is catalytically inactive. In an attempt to establish a chemical **model** for the reduction of the conjugated imine such as DELTA-3,4-glucosene intermediate by thiols, kinetics of the reduction of DCPIP by cysteine was examined.

L37 ANSWER 68 OF 94 MEDLINE on STN DUPLICATE 28
TI A **model** for the role of multiple cysteine residues involved in ribonucleotide reduction: amazing and still confusing.
SO Biochemistry, (1992 Oct 13) 31 (40) 9733-43.
Journal code: 0370623. ISSN: 0006-2960.
AU Mao S S; Holler T P; Yu G X; Bollinger J M Jr; Booker S; Johnston M I; Stubbe J
AB Ribonucleotide reductase from Escherichia **coli** catalyzes the conversion of nucleotides to deoxynucleotides. Multiple cysteins have been postulated to play a key role in this process. To test the role of

various cysteines in nucleotide reduction, a variety of single and double mutants of the R1 subunit were prepared: C754S, C759S, C754-759S, C462S, C462A, C230S, and C292S. Due to the expression system, each mutant contains small amounts of contaminating wt-R1 (estimated to be 1.5-3% based on activity). An epitope tagging method in conjunction with anion exchange chromatography was used to partially resolve the mutant R1 from the wt-R1. The interaction of these mutants with the normal substrate was studied, which allowed a **model** to be proposed in which five cysteines of the R1 subunit of RDPR play a role in catalysis. C754S and C759S R1s catalyze CDP formation at rates similar to wt-R1 when DTT is used as a reductant. However, when thioredoxin (TR)/**thioredoxin reductase** (TRR)/NADPH is used as reductant, the rates of dNDP production are similar to those expected for contaminating wt-R1 present as a heterodimer with the mutant. The impaired nature of these mutants with respect to reduction by TR suggests that their function is to transfer reducing equivalents from TR to the active site disulfide of R1 produced during NDP reduction. Single-turnover experiments, designed to avoid the problem of contaminating wt-R1, also support this role for C754 and C759. The double serine mutant of 754 and 759 has catalytic activity with DTT that is one-third the rate of wt-R1 with thioredoxin. C225 and C462 are thought to be the active site cysteines oxidized concomitantly with NDP reduction. Conversion of these cysteines to serines results in R1 mutants which convert the normal substrate into a mechanism-based inhibitor. C462SR1 upon incubation with R2 and [3'-3H,U-14C]UDP results in uracil release, 3H2O production, 3H,14C-labeled protein which has an absorbance change at 320 nm, and slow loss of the tyrosyl radical on R2. The isotope effect (kH/k3H) on 3' carbon-hydrogen bond cleavage is 1.7. This sequence of events is independent of the reductant, consistent with the postulate that C462 is an active site thiol. The C462AR1 has properties similar to C462SR1. Several additional mutant R1s, C230SR1, and C292SR1 were shown to have activities similar to wt-R1 with both TR/TRR/NADPH and DTT.

L37 ANSWER 69 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 29

TI THIOREDOXIN REGENERATES PROTEINS INACTIVATED BY OXIDATIVE STRESS IN ENDOTHELIAL-CELLS

SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (01 NOV 1992) Vol. 209, No. 3, pp. 917-922.
ISSN: 0014-2956.

AU FERNANDO M R (Reprint); NANRI H; YOSHITAKE S; NAGATAKUNO K; MINAKAMI S

AB The thioredoxin/**thioredoxin reductase** system has been studied as regenerative machinery for proteins inactivated by oxidative stress in vitro and in cultured endothelial cells. Mammalian glyceraldehyde-3-phosphate dehydrogenase was used as the main **model** enzyme for monitoring the oxidative damage and the regeneration. Thioredoxin and its reductase purified from bovine liver were used as the regenerating system. The physiological concentrations (2 - 14 muM) of reduced thioredoxin, with 0.125 muM **thioredoxin reductase** and 0.25 mM NADPH, regenerated H2O2-inactivated glyceraldehyde-3-phosphate dehydrogenase and other mammalian enzymes almost completely within 20 min at 37-degrees-C. Although the treatment of endothelial cells with 0.2 - 12 mM H2O2 for 5 min resulted in a marked decrease in the activity of glyceraldehyde-3-phosphate dehydrogenase, it had no effect on the activities of thioredoxin and **thioredoxin reductase**. Essentially all of the thioredoxin in endothelial cells at control state was in the reduced form and 70 - 85 % remained in the reduced form even after the H2O2 treatment. The inactivated glyceraldehyde-3-phosphate dehydrogenase in a cell lysate prepared from the H2O2-treated endothelial cells was regenerated by incubating the lysate with 3 mM NADPH at 37-degrees-C and the antiserum raised against bovine liver thioredoxin inhibited the regeneration. The inhibition of **thioredoxin reductase** activity by 13-cis-retinoic acid resulted in a decrease in the regeneration of glyceraldehyde-3-phosphate dehydrogenase in the H2O2-treated endothelial cells. The present findings provide evidence that thioredoxin is involved in the regeneration of proteins inactivated by oxidative stress in endothelial cells.

L37 ANSWER 70 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI MOLECULAR-CLONING AND ANALYSIS OF THE GENE ENCODING THE NADH OXIDASE FROM STREPTOCOCCUS-FAECALIS 10C1 - COMPARISON WITH NADH PEROXIDASE AND THE FLAVOPROTEIN DISULFIDE REDUCTASES
SO JOURNAL OF MOLECULAR BIOLOGY, (05 OCT 1992) Vol. 227, No. 3, pp. 658-671. ISSN: 0022-2836.
AU ROSS R P (Reprint); CLAIBORNE A

L37 ANSWER 71 OF 94 MEDLINE on STN
TI Mutation of conserved residues in Escherichia **coli** thioredoxin: effects on stability and function.
SO Protein science : a publication of the Protein Society, (1992 May) 1 (5) 609-16.
Journal code: 9211750. ISSN: 0961-8368.
AU Gleason F K
AB Mutations were made in three highly conserved residues in Escherichia **coli** thioredoxin. An internal charged residue, Asp-26, was changed to an alanine. The mutant protein was more stable than the wild type. It can function as a substrate for **thioredoxin reductase** with a 10-fold increase in the Km over the wild type. Although the redox potential was not substantially changed from that of the wild type, thioredoxin D26A was a poor reducing agent for ribonucleotide reductase. Asp-26 apparently serves to maintain an optimal charge distribution in the active site region for interaction with other proteins. Mutation of a surface Pro-34 in the active site disulfide ring to a serine had little effect on protein stability. A slight decrease in the redox potential (9 mV) made thioredoxin P34S a better reducing agent for ribonucleotide reductase. In contrast, mutation of the internal cis Pro-76 to an alanine destabilized the protein. The data indicate a change had also occurred in the charge distribution in the active site region. Thioredoxin P76A had a higher redox potential than the wild type protein and was not an effective reducing agent for ribonucleotide reductase. It was concluded that this residue is essential for maintaining the conformation of the active site and the redox potential of thioredoxin.

L37 ANSWER 72 OF 94 MEDLINE on STN
TI Stimulation of the dithiol-dependent reductases in the vitamin K cycle by the thioredoxin system. Strong synergistic effects with protein disulphide-isomerase.
SO Biochemical journal, (1992 Jan 1) 281 (Pt 1) 255-9.
Journal code: 2984726R. ISSN: 0264-6021.
AU Soute B A; Groenen-van Dooren M M; Holmgren A; Lundstrom J; Vermeer C
AB It has been shown previously that the thioredoxin system (thioredoxin + **thioredoxin reductase** + NADPH) may replace dithiothreitol (DTT) as a cofactor for vitamin KO and K reductase in salt-washed detergent-solubilized bovine liver microsomes. Here we demonstrate that the system can be improved further by adding protein disulphide-isomerase (PDI) to the components mentioned above. Moreover, NADPH may be replaced by reduced RNAase as a hydrogen donor. In our in vitro system the various protein cofactors were required at concentrations 2-5 orders of magnitude lower than that of DDT, whereas the maximal reaction rate was about 3-fold higher. PDI stimulated the thioredoxin-driven reaction about 10-fold, with an apparent Km value of 8 microM. These data suggest that in the vitro system the formation of disulphide bonds is somehow linked to the vitamin K-dependent carboxylation of glutamate residues. In vivo, both disulphide formation and vitamin K-dependent carboxylation are post-translational modifications taking place at the luminal side of the endoplasmic reticulum of mammalian secretory cells. The possibility that the reactions are also coupled in vivo is discussed.

L37 ANSWER 73 OF 94 CAPLUS COPYRIGHT 2004 ACS on STN
TI Mimicking the active site of protein disulfide-isomerase by substitution of proline 34 in Escherichia **coli** thioredoxin
SO Journal of Biological Chemistry (1991), 266(15), 9494-500
CODEN: JBCHA3; ISSN: 0021-9258
AU Krause, Gunter; Lundstroem, Johanna; Lopez Barea, Juan; Pueyo de la Cuesta, Carmen; Holmgren, Arne
AB To mimic the active sites (Trp-Cys-Gly-His-Cys) contained in two thioredoxin-like domains of the eukaryotic enzyme protein disulfide-isomerase (PDI, EC 5.3.4.1), the Pro-34 residue of E. **coli** thioredoxin (Trx) was replaced by His (histidine) using

site-directed mutagenesis. The mutant P34H Trx was isolated in high yield and was stable. The equil. between Trx and NADPH in the **thioredoxin reductase** (TR)-catalyzed reaction revealed that the redox potential (E0') of P34H Trx at pH 7.0 was-235 mV as compared with -270 mV for wild type (wt) Trx. The higher E0' value made P34H Trx more similar to PDI and contributed to prominent changes in Trx functions, e.g. improved activity with TR and slower redn. of protein disulfides. Compared to wt Trx, the P34H oxidized Trx was about twice as good a substrate for TR from E. **coli** and four times as efficient with calf thymus TR. A novel fluorimetric assay permitted direct recording of the reaction between insulin disulfide(s) and reduced Trx. At pH 8 and 15.degree., second-order rate consts. for wt Trx of 2 .times. 104 M-1 and for P34H Trx of 3 .times. 103 M-1 s-1 were obtained, and a different equil. was obsd. consistent with differences in E0' values. Also when the redn. mechanism of insulin was examd. using NADPH and TR, P34H Trx behaved differently from wt Trx or PDI. P34H Trx may be useful as an analog of PDI for disulfide formation in vivo and in vitro.

L37 ANSWER 74 OF 94 MEDLINE on STN
TI Substitution of the conserved tryptophan 31 in Escherichia **coli** thioredoxin by site-directed mutagenesis and structure-function analysis.
SO Journal of biological chemistry, (1991 Mar 5) 266 (7) 4056-66.
Journal code: 2985121R. ISSN: 0021-9258.
AU Krause G; Holmgren A
AB All prokaryotic and eukaryotic thioredoxins contain a conserved tryptophan residue, exposed at the active site disulfide/dithiol. The role of this W31 in Escherichia **coli** thioredoxin (Trx) was studied by site-directed mutagenesis. Four mutant Trx with W31Y, W31F, W31H, and W31A replacements were characterized. Very low tryptophan fluorescence emission from the remaining W28 was observed in all mutant Trx; reduction resulted in large, but variable increases (up to 11-fold) of fluorescence, to levels higher than in native or denatured wild-type Trx, demonstrating a previously postulated change involving W28. All W31 mutant Trx were good substrates for E. **coli thioredoxin reductase**. Compared with wild type, the apparent Km values were increased less than 2-fold for the W31A, W31H, and W31F Trx and the W31Y Trx showed even slightly higher catalytic efficiency (kcat/Km value). Functions of reduced Trx with ribonucleotide reductase and in reduction of insulin disulfides were more strongly influenced by the W31 replacements, in particular at low pH for A and H residues. T7 DNA polymerase activity generated by T7 gene 5 protein and reduced Trx was lowered by large factors for W31Y, W31A, or W31H compared with W31F or the wild-type protein. The in vivo function of Trx was studied by using pUC118-trxA expression in an E. **coli** trxA- background. The trxA genes with W31Y and W31F substitutions restored, fully and partly, the methionine sulfoxide utilization of a trxA- metE- test strain; W31A and W31H mutations resulted in no growth. Propagation of M13 was moderately impeded by W31Y and W31F or severely by W31A and W31H replacements. Growth of a phage T3/7 hybrid was possible only with the W31Y and W31F substitutions reflecting the in vitro results for T7 DNA polymerase.

L37 ANSWER 75 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI STRUCTURE OF NADH PEROXIDASE FROM STREPTOCOCCUS-FAECALIS 10C1 REFINED AT 2.16 A RESOLUTION
SO JOURNAL OF MOLECULAR BIOLOGY, (1991) Vol. 221, No. 4, pp. 1325-1344.
AU STEHLE T (Reprint); AHMED S A; CLAIBORNE A; SCHULZ G E

L37 ANSWER 76 OF 94 MEDLINE on STN DUPLICATE 30
TI Convergent evolution of similar function in two structurally divergent enzymes.
SO Nature, (1991 Jul 11) 352 (6331) 172-4.
Journal code: 0410462. ISSN: 0028-0836.
AU Kuriyan J; Krishna T S; Wong L; Guenther B; Pahler A; Williams C H Jr; Model P
AB An example of two related enzymes that catalyse similar reactions but possess different active sites is provided by comparing the structure of Escherichia **coli thioredoxin reductase** with glutathione reductase. Both are dimeric enzymes that catalyse the reduction of disulphides by pyridine nucleotides through an enzyme disulphide and a flavin. Human glutathione reductase contains four

structural domains within each molecule: the flavin-adenine dinucleotide (FAD)- and nicotinamide-adenine dinucleotide phosphate (NADPH)-binding domains, the 'central' domain and the C-terminal domain that provides the dimer interface and part of the active site. Although both enzymes share the same catalytic mechanism and similar tertiary structures, their active sites do not resemble each other. We have determined the **crystal** structure of E. **coli thioredoxin reductase** at 2 A resolution, and show that **thioredoxin reductase** lacks the domain that provides the dimer interface in glutathione reductase, and forms a completely different dimeric structure. The catalytically active disulphides are located in different domains on opposite sides of the flavin ring system. This suggests that these enzymes diverged from an ancestral nucleotide-binding protein and acquired their disulphide reductase activities independently.

L37 ANSWER 77 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI CONVERGENT EVOLUTION OF SIMILAR FUNCTION IN 2 STRUCTURALLY DIVERGENT ENZYMES

SO NATURE, (1991) Vol. 352, No. 6331, pp. 172-174.

AU KURIYAN J (Reprint); KRISHNA T S R; WONG L; GUENTHER B; PAHLER A; WILLIAMS C H; MODEL P

AB AN example of two related enzymes that catalyse similar reactions but possess different active sites is provided by comparing the structure of Escherichia **coli thioredoxin reductase** with glutathione reductase 1. Both are dimeric enzymes that catalyse the reduction of disulphides by pyridine nucleotides through an enzyme disulphide and a flavin 2. Human glutathione reductase contains four structural domains within each molecule: the flavin-adenine dinucleotide (FAD)- and nicotinamide-adenine dinucleotide phosphate (NADPH)-binding domains, the 'central' domain and the C-terminal domain that provides the dimer interface and part of the active site 3,4. Although both enzymes share the same catalytic mechanism and similar tertiary structures, their active sites do not resemble each other 5,6. We have determined the **crystal** structure of E. **coli thioredoxin reductase** at 2 angstrom resolution, and show that **thioredoxin reductase** lacks the domain that provides the dimer interface in glutathione reductase, and forms a completely different dimeric structure. The catalytically active disulphides are located in different domains on opposite sides of the flavin ring system. This suggests that these enzymes diverged from an ancestral nucleotide-binding protein and acquired their disulphide reductase activities independently.

L37 ANSWER 78 OF 94 MEDLINE on STN

TI Characterization of Escherichia **coli** thioredoxins with altered active site residues.

SO Biochemistry, (1990 Apr 17) 29 (15) 3701-9.
Journal code: 0370623. ISSN: 0006-2960.

AU Gleason F K; Lim C J; Gerami-Nejad M; Fuchs J A

AB Escherichia **coli** thioredoxin is a small disulfide-containing redox protein with the active site sequence Cys-Gly-Pro-Cys-Lys. Mutations were made in this region of the thioredoxin gene and the mutant proteins expressed in E. **coli** strains lacking thioredoxin. Mutant proteins with a 17-membered or 11-membered disulfide ring were inactive in vivo. However, purified thioredoxin with the active site sequence Cys-Gly-Arg-Pro-Cys-Lys is still able to serve as a substrate for **thioredoxin reductase** and a reducing agent in the ribonucleotide reductase reaction, although with greatly reduced catalytic efficiency. A smaller disulfide ring, with the active site sequence Cys-Ala-Cys, does not turn over at a sufficient rate to be an effective reducing agent. Strain in the small ring favors the formation of intermolecular disulfide bonds. Alteration of the invariant proline to a serine has little effect on redox activity. The function of this residue may be in maintaining the stability of the active site region rather than participation in redox activity or protein-protein interactions. Mutation of the positively charged lysine in the active site to a glutamate residue raises the Km values with interacting enzymes. Although it has been proposed that the positive residue at position 36 is conserved to maintain the thiolate anion on Cys-32 (Kallis & Holmgren, 1985), the presence of the negative charge at this position does not alter the pH dependence of activity or fluorescence behavior. The lysine is most likely conserved to

facilitate thioredoxin-protein interactions.

L37 ANSWER 79 OF 94 MEDLINE on STN
TI Modifications of the active center of T4 thioredoxin by site-directed mutagenesis.
SO Journal of biological chemistry, (1990 Feb 25) 265 (6) 3183-8.
Journal code: 2985121R. ISSN: 0021-9258.
AU Joelson T; Sjoberg B M; Eklund H
AB The active site sequence of T4 thioredoxin, Cys-Val-Tyr-Cys, has been modified in two positions to Cys-Gly-Pro-Cys to mimic that of Escherichia **coli** thioredoxin. The two point mutants Cys-Gly-Tyr-Cys and Cys-Val-Pro-Cys have also been constructed. The mutant proteins have similar reaction rates with T4 ribonucleotide reductase as has the wild-type T4 thioredoxin. Mutant T4 thioredoxins with Pro instead of Tyr at position 16 in the active site sequence have three to four times lower apparent KM with E. **coli** ribonucleotide reductase than wild-type T4 thioredoxin. The KM values for these mutant proteins which do not have Tyr in position 16 are thus closer to E. **coli** thioredoxin than to the wild-type T4 thioredoxin. The bulky tyrosine side chain probably prevents proper interactions to E. **coli** ribonucleotide reductase. Also the redox potentials of these two mutant thioredoxins are lower than that of the wild-type T4 thioredoxin and are thereby more similar to the redox potential of E. **coli** thioredoxin. Mutations in position 15 behave more or less like the wild-type protein. The kinetic parameters with E. **coli thioredoxin reductase** are similar for wild-type and mutant T4 thioredoxins except that the apparent kcat is lower for the mutant protein with Pro instead of Tyr in position 16. The active site sequence of T4 thioredoxin has also been changed to Cys-Pro-Tyr-Cys to mimic that of glutaredoxins. This change does not markedly alter the reaction rate of the mutant protein with T4 ribonucleotide reductase or E. **coli thioredoxin reductase**, but the redox potential is lower for this mutant protein than for wild-type T4 thioredoxin.

L37 ANSWER 80 OF 94 MEDLINE on STN DUPLICATE 31
TI Crystallization and preliminary **x-ray** characterization of **thioredoxin reductase** from Escherichia **coli**
.
SO Journal of biological chemistry, (1989 Aug 5) 264 (22) 12752-3.
Journal code: 2985121R. ISSN: 0021-9258.
AU Kuriyan J; Wong L; Russel M; Model P
AB Single **crystals** of **thioredoxin reductase**, suitable for **x-ray** diffraction studies, have been obtained at room temperature by vapor diffusion of 10-20 mg/ml protein solution against 35% polyethylene glycol containing 200 mM ammonium sulfate. Good quality **crystals** appear spontaneously only from a protein solution that had been stored for more than a year at 4 degrees C, although large single **crystals** are reproducibly obtained from fresh protein solutions by micro-seeding. The space group is P6(3)22 (a = b = 123.8 A, c = 81.6 A), with one monomer of the enzyme (34.5 kDa) in the crystallographic asymmetric unit. The **crystals** are well ordered and diffract to beyond 2 A resolution.

L37 ANSWER 81 OF 94 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI CRYSTALLIZATION AND PRELIMINARY-**X-RAY** CHARACTERIZATION OF **THIOREDOXIN REDUCTASE** FROM ESCHERICHIA-**COLI**
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1989) Vol. 264, No. 22, pp. 12752-12753.
AU KURIYAN J (Reprint); WONG L; RUSSEL M; MODEL P

L37 ANSWER 82 OF 94 MEDLINE on STN
TI The role of thioredoxin in filamentous phage assembly. Construction, isolation, and characterization of mutant thioredoxins.
SO Journal of biological chemistry, (1986 Nov 15) 261 (32) 14997-5005.
Journal code: 2985121R. ISSN: 0021-9258.
AU Russel M; Model P
AB Filamentous phage assembly in vivo shows an absolute requirement for thioredoxin and a partial requirement for **thioredoxin reductase**. Mutants in which one or both of the active site cysteine residues of thioredoxin were changed to alanine or serine were constructed and shown to support filamentous phage assembly. Some of the

mutants were almost as effective as wild-type thioredoxin, while others supported phage assembly only when high levels of the mutant protein were present in the infected cell. The mutant proteins were all inactive in an assay which couples oxidation of NADPH to reduction of 5,5'-dithiobis-2-nitrobenzoic acid) via **thioredoxin reductase** and thioredoxin. These active site mutants make phage assembly completely independent of **thioredoxin reductase**, which suggests that the phage needs, and the active site mutants provide, the proteins in the reduced conformation. Other mutants were isolated on the basis of their failure to support filamentous phage growth. These specified mutant thioredoxin proteins with varying levels of redox activity in vivo and in vitro. The locations of these mutations suggest that the surface of thioredoxin thought to interact with **thioredoxin reductase** also interacts with the filamentous phage assembly machinery. An in vivo assay for thioredoxin redox function, based on the ability of cells to utilize methionine sulfoxide, was developed. Met- cells containing mutant thioredoxins that are inactive in vitro do not form colonies on plates containing methionine sulfoxide as the sole methionine source.

L37 ANSWER 83 OF 94 MEDLINE on STN DUPLICATE 32
TI Thioredoxin is required for filamentous phage assembly.
SO Proceedings of the National Academy of Sciences of the United States of America, (1985 Jan) 82 (1) 29-33.
Journal code: 7505876. ISSN: 0027-8424.
AU Russel M; Model P
AB Sequence comparisons show that the fip gene product of Escherichia **coli**, which is required for filamentous phage assembly, is thioredoxin. Thioredoxin serves as a cofactor for reductive processes in many cell types and is a constituent of phage T7 DNA polymerase. The fip-1 mutation makes filamentous phage and T7 growth temperature sensitive in cells that carry it. The lesion lies within a highly conserved thioredoxin active site. **Thioredoxin reductase** (NADPH), as well as thioredoxin, is required for efficient filamentous phage production. Mutant phages defective in phage gene I are particularly sensitive to perturbations in the fip-thioredoxin system. A speculative **model** is presented in which **thioredoxin reductase**, thioredoxin, and the gene I protein interact to drive an engine for filamentous phage assembly.

L37 ANSWER 84 OF 94 MEDLINE on STN DUPLICATE 33
TI Mechanism of inactivation of Escherichia **coli** and Lactobacillus leichmannii ribonucleotide reductases by 2'-chloro-2'-deoxynucleotides: evidence for generation of 2-methylene-3(2H)-furanone.
SO Biochemistry, (1984 Oct 23) 23 (22) 5214-25.
Journal code: 0370623. ISSN: 0006-2960.
AU Harris G; Ator M; Stubbe J
AB Incubation of 2'-chloro-2'-deoxy[3'-3H]uridine 5'-diphosphate ([3'-3H]ClUDP) with Escherichia **coli** ribonucleotide reductase (RDPR) and use of thioredoxin-**thioredoxin reductase** as reductants result in release of 4.7 equiv of 3H2O/equiv of B1 protomer, concomitant with enzyme inactivation. Inactivation is accompanied by the production of 6 equiv of inorganic pyrophosphate [Stubbe, J. A., & Kozarich, J.W. (1980) J. Am. Chem. Soc. 102, 2505-2507] and by the release of uracil as previously shown [Thelander, L., Larsson, A., Hobbs, J., & Eckstein, F. (1976) J. Biol. Chem. 251, 1398-1405]. Reisolation of RDPR by Sephadex chromatography and analysis by scintillation counting indicate that 0.96 equiv of 3H is bound per protomer of the B1 subunit of the inactivated enzyme. Incubation of [5'-3H]ClUDP with RDPR followed by similar analysis indicates that 4.6 mol of 3H is bound per protomer of the B1 subunit of the inactivated enzyme. No 3H2O is released, and 6 equiv of inorganic pyrophosphate is produced during the inactivation. RDPR is protected against inactivation when dithiothreitol (DTT) is used as a reductant in place of thioredoxin-**thioredoxin reductase**. Incubation of [5'-3H]ClUDP with RDPR and DTT results in the isolation of CHCl3-extractable material that exhibits infrared absorptions at 1710 and 1762 cm-1. The infrared spectrum and the **NMR** spectrum of the CHCl3-extracted material are very similar to **model** compounds prepared by the interaction of 2-methylene-3(2H)-furanone with ethanethiol. Incubation of ribonucleoside-triphosphate reductase (RTPR) from Lactobacillus leichmannii with [3'-3H]ClUTP and 3 mM DTT also results

in time-dependent 3H2O release concomitant with enzyme inactivation. Reisolation of the inactive protein by Sephadex chromatography followed by radiochemical analysis indicates that 0.4 equiv of 3H is bound covalently per mol of inactivated enzyme. Similar studies with [5'-3H]ClUTP indicate that 2.9 equiv of 3H is bound covalently per mol of inactivated enzyme. No 3H2O is released. High concentrations of DTT protect the enzyme against inactivation. Extraction of the enzymatic reaction mixture with CHCl3 and analysis of the isolated products result in an infrared spectrum and an **NMR** spectrum remarkably similar to those observed with the E. **coli** RDPR. Data presented are consistent with the proposal that both the E. **coli** and L. leichmannii enzymes are able to catalyze the breakdown of the appropriate 2'-chloro-2'-deoxynucleotide to a 3'-keto-2'-deoxynucleotide that can collapse to form the reactive sugar intermediate 2-methylene-3(2H)-furanone.(ABSTRACT TRUNCATED AT 400 WORDS)

L37 ANSWER 85 OF 94 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI THE ROLE OF THIOREDOXIN IN THE OXIDATION-REDUCTION CELL METABOLISM.
SO Biologicke Listy, (1984) Vol. 49, No. 2, pp. 137-146.
CODEN: BILIAC. ISSN: 0366-0486.
AU SLABY I [Reprint author]
AB Thioredoxin was identified as a part of an enzyme system from Escherichia **coli** required for the biosynthesis of deoxyribonucleotides. It is a small (MW 11,700), ubiquitous electron-transport protein. The functional group of thioredoxin consists of a single cystine residue that in the oxidized protein (thioredoxin-S2) forms a disulfide bridge of defined structure. The specific enzyme **thioredoxin reductase** catalyzes in vitro the reduction of thioredoxin by NADPH, which generates the reduced form (thioredoxin-(SH)2). Thioredoxin-(SH)2 is a potent biological reductant. Apart from its function in DNA synthesis as H donor for ribonucleotide reductase, it has also been shown to be involved in the enzyme reduction of methionine sulfoxide and of sulfate in yeast. Thioredoxin-(SH)2 spontaneously reduces disulfide bonds in proteins such as insulin, fibrinogen, oxytocin, human choriogonadotropin, and will thus be of importance for the overall thioldisulfide status in the cell. A completely new role for thioredoxin in DNA synthesis was discovered. E. **coli** thioredoxin wsa found to be a subunit of a phage T7-induced DNA polymerase, detected after virus infection. Phage T7 DNA polymerase is composed of 1 phage-coded gene 5 protein and 1 molecule of bacterial thioredoxin. The specific function of thioredoxin in this unique enzyme is presently unknown. The structure of thioredoxin-S2 from E. **coli** was determined by **X-ray** crystallography to a resolution of 0.28 nm. The molecule contains 108 amino acid residues, most of which are located in the form of secondary structure elements, consisting of a central core of a 5-strand .beta.-pleated sheet surrounded by 4 .alpha.-helices. The oxidation-reduction functional disulfide bond which is formed from cysteine-32 and cysteine-35 is located in an elevated part of the the molecule.

L37 ANSWER 86 OF 94 MEDLINE on STN
TI Proton stoichiometry in the reduction of the FAD and disulfide of Escherichia **coli thioredoxin reductase**.
Evidence for a base at the active site.
SO Journal of biological chemistry, (1983 Nov 25) 258 (22) 13795-805.
Journal code: 2985121R. ISSN: 0021-9258.
AU O'Donnell M E; Williams C H Jr
AB The oxidation-reduction midpoint potentials, Em, of the FAD and active site disulfide couples of Escherichia **coli thioredoxin reductase** have been determined from pH 5.5 to 8.5. The FAD and disulfide couples have similar Em values and thus a linked equilibrium of four microscopic enzyme oxidation-reduction states exists. The binding of phenylmercuric acetate to one enzyme form could be monitored which allowed solving the four microscopic Em values. The Em values at pH 7.0 and 12 degrees C of the four couples of **thioredoxin reductase** are: (S)2-enzyme-FAD/FADH2 = -0.243 V, (SH)2-enzyme-FAD/FADH2 = -0.260 V, (FAD)-enzyme-(S)2/(SH)2 = -0.254 V, and (FADH2)-enzyme-(S)2/(SH)2 = -0.271 V. Thus, at pH 7.0, the FAD and disulfide moieties have a 0.017-V negative interaction and Em values which are different by 0.011 V. The delta Em/delta pH of the FAD couples E2m and E3m are about 0.060 V/pH

throughout the pH range studied, showing an approximately 2-proton stoichiometry of reduction of the enzyme FAD. The delta Em/delta pH of the disulfide couples E1m and E4m are about 0.052 V/pH from pH 5.5 to 8.5, showing an apparently nonintegral proton stoichiometry of reduction of 1.8 in this pH range. This proton stoichiometry suggests the presence of a base with an ionization behavior that is linked to the oxidation-reduction state of the disulfide. A novel method is presented for determining the pK values on oxidized and reduced enzyme which agrees with the less accurate classical method. The proton stoichiometry results are consistent with the presence of a thiol-base ion pair in which the pK of the base is elevated from 7.6 in disulfide containing enzyme to greater than 8.5 upon forming an ion pair with a thiol anion of pK 7.0 generated upon reduction of the disulfide. The fluorescence of the FAD in **thioredoxin reductase** decreases as the pH is lowered with a pK of 7.0, direct evidence for a base near the FAD probably distinct from the base interacting with the dithiol.

L37 ANSWER 87 OF 94 MEDLINE on STN
TI A mutant thioredoxin from Escherichia **coli** tsnC 7007 that is nonfunctional as subunit of phage T7 DNA polymerase.
SO Journal of biological chemistry, (1981 Mar 25) 256 (6) 3118-24. Journal code: 2985121R. ISSN: 0021-9258.
AU Holmgren A; Kallis G B; Nordstrom B
AB Thioredoxin was purified to homogeneity from the Escherichia **coli** mutant tsnC 7007 that is defective in phage T7 DNA replication and previously shown to contain a missense thioredoxin. Tryptic peptide maps of reduced and carboxymethylated 7007 thioredoxin combined with amino acid sequence analysis revealed one amino acid substitution; Gly-92 in thioredoxin is exchanged to an aspartic acid residue in the 7007 protein. The missense thioredoxin gave no activity with the gene 5 protein of phage T7 in the complementation to active T7 DNA polymerase. It competitively inhibited the complementation of wild type thioredoxin and gene 5 protein and formed a complex with the gene 5 protein that was retained by antithioredoxin Sepharose. The 7007 thioredoxin has reduced catalytic activity with **thioredoxin reductase**, ribonucleotide reductase, or as a protein disulfide reductase. The apparent Km value of 7007 thioredoxin as a substrate for **thioredoxin reductase** was increased 3-fold relative to normal thioredoxin, and the Vmax value was decreased 7-fold. The position of GLy-92 in the known three-dimensional structure of thioredoxin-S2 is correlated with the changed functional properties of the substituted mutant protein.

L37 ANSWER 88 OF 94 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI THE FERREDOXIN THIO REDOXIN SYSTEM OF ENZYME REGULATION IN A CYANOBACTERIUM NOSTOC-MUSCORUM.
SO Archives of Microbiology, (1981) Vol. 130, No. 1, pp. 14-18. CODEN: AMICCW. ISSN: 0302-8933.
AU YEE B C [Reprint author]; DE LA TORRE A; CRAWFORD N A; LARA C; CARLSON D E; BUCHANAN B B
AB Cell-free preparations of the cyanobacterium (blue-green alga) N. muscorum were assayed for thioredoxins and enzymes catalyzing the ferredoxin and NADP-linked reduction of thioredoxin. Nostoc had 2 different thioredoxins: one of approximate MW 16,000 (designated Nostoc thioredoxin f) that selectively activated chloroplast fructose 1,6-bisphosphatase and another of approximate MW 9000 (designated Nostoc thioredoxin m) that selectively activated chloroplast NADP-malate dehydrogenase. The 2 thioredoxins could be reduced either chemically with dithiothreitol or photochemically with ferredoxin and ferredoxin-**thioredoxin reductase** which, like the recently found regulatory Fe-S protein ferralterin, was present in Nostoc cells. Nostoc ferredoxin-**thioredoxin reductase** appeared to be similar to its chloroplast counterpart in enzyme specificity, MW and spectral properties. The Nostoc and spinach chloroplast ferredoxin-**thioredoxin reductases** as well as their thioredoxins, ferredoxins and chlorophyll containing membranes were interchangeable in activating chloroplast fructose 1,6-bisphosphatase and NADP-malate dehydrogenase. There was no evidence of an NADP-linked **thioredoxin reductase** such as that of E. **coli**. Apparently, the cyanobacteria resemble higher plants in having a function ferredoxin/thioredoxin system rather than an NADP/thioredoxin system

typical of other bacteria.

L37 ANSWER 89 OF 94 MEDLINE on STN
TI Pyridine nucleotide - disulfide oxidoreductases.
SO Experientia. Supplementum, (1980) 36 149-80. Ref: 115
Journal code: 0320043. ISSN: 0071-335X.
AU Holmgren A

L37 ANSWER 90 OF 94 MEDLINE on STN DUPLICATE 34
TI Thioredoxin-C': mechanism of noncovalent complementation and reactions of the refolded complex and the active site containing fragment with **thioredoxin reductase**.
SO Biochemistry, (1979 Dec 11) 18 (25) 5591-9.
Journal code: 0370623. ISSN: 0006-2960.
AU Holmgren A; Slaby I

L37 ANSWER 91 OF 94 MEDLINE on STN
TI Three-dimensional structure of thioredoxin induced by bacteriophage T4.
SO Proceedings of the National Academy of Sciences of the United States of America, (1978 Dec) 75 (12) 5827-30.
Journal code: 7505876. ISSN: 0027-8424.
AU Soderberg B O; Sjoberg B M; Sonnerstam U; Branden C I
AB The three-dimensional structure of thioredoxin from bacteriophage T4 has been determined from a 2.8-angstrom resolution electron density map. Phase angles for this map were determined from one heavy atom derivative and anomalous differences from cadmium in the native **crystals**. The molecule of 87 amino acid residues is built up from two simple folding units; a betaalphabeta unit from the amino end of the chain and a betabetaalpha unit from the carboxyl end. This structure is similar to that of thioredoxin from Escherichia **coli** in spite of their completely different amino acid sequences. The redox-active S--S bridge is part of a protrusion of the molecule as in E. **coli** thioredoxin, but with quite different surroundings. The structural differences in this region have been correlated to differences in specificity towards the enzyme ribonucleotide reductase from different species.

L37 ANSWER 92 OF 94 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Purification and characterization of yeast **thioredoxin reductase**.
SO Biochimica et Biophysica Acta, (1974) 327/2 (274-281).
CODEN: BBACAQ
AU Speranza M.L.; Ronchi S.; Minchiotti L.

L37 ANSWER 93 OF 94 MEDLINE on STN
TI Studies on **thioredoxin reductase** from Escherichia **coli** B. The relation of structure and function.
SO European journal of biochemistry / FEBS, (1968 Apr) 4 (3) 407-19.
Journal code: 0107600. ISSN: 0014-2956.
AU Thelander L

L37 ANSWER 94 OF 94 CAPLUS COPYRIGHT 2004 ACS on STN
TI Enzymic synthesis of deoxyribonucleotides. IV. Isolation and characterization of thioredoxin, the hydrogen donor from Escherichia **coli** B
SO Journal of Biological Chemistry (1964), 239(10), 3436-44
CODEN: JBCHA3; ISSN: 0021-9258
AU Laurent, Torvard C.; Moore, E. Colleen; Reichard, Peter
AB cf. CA 59, 10388b. A protein, called thioredoxin, was purified from E. **coli** B which, in the presence of reduced triphosphopyridine nucleotide (TPNH) can replace reduced lipoate as the H donor in the reductive formation of deoxycytidine diphosphate from cytidine diphosphate (CDP) with the CDP-reductase system from E. **coli**. Thioredoxin was obtained in an apparently pure form, with a mol. weight of .apprx.12,000 as judged from amino acid compn., ultracentrifugal data, and enzymic activity. It was homogeneous on microelectrophoresis on agarose between pH 4 and 8.6. Thioredoxin consists of a single polypeptide chain with NH2-terminal serine and contains one mol. of cystine. The SS bond can be reduced either chem. (e.g. with mercaptoethylamine) or by a specific enzyme (**thioredoxin reductase**) with TPNH.

The partially purified CDP-reductase system from E. **coli** contains **thioredoxin reductase**. Evidence is presented that reduced thioredoxin(SH)2 is the actual H donor in the redn. of CDP and that reduced lipoate acts as a **model** substance. In the presence of an excess of TPNH (and **thioredoxin reductase**), thioredoxin acts in a catalytic fashion.

=> log y